0022-1767/92/1487-2154$02.00/0
THE JOURNAL OF IMMUNOLOGY

Vol. 148, 2154-2158, No. 7, April 1, 1992
Printed in U.S.A.

AN ASSOCIATED MOLECULE, p64, WITH IL-2 RECEPTOR β CHAIN

Its Possible Involvement in the Formation of the Functional Intermediate-Affinity IL-2 Receptor Complex[1]

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We identified previously a membrane molecule, p64, which co-precipitates with the IL-2R β-chain in human T cells. We have now investigated the biologic significance of p64 in the formation of the functional IL-2R complex with cell lines transfected with cDNA of IL-2Rα- and/or β-chains. Two functional parameters associated with IL-2R, IL-2 binding ability and association of p64 with the β-chain, were examined. Two subclones, MOLTβ-11 and MOLTβ-12, of an IL-2Rβ cDNA-transfected MOLT4 clone expressed similar numbers of IL-2Rβ molecules on cell surfaces and bound to IL-2 with intermediate affinity. However, the numbers of IL-2 binding sites were significantly lower than those of IL-2Rβ molecules and considerably different between the two subclones. The amount of p64 co-precipitated with IL-2Rβ was proportional to numbers of the IL-2 binding sites in the two subclones. In addition, neither p64 co-precipitation nor IL-2 binding was detected in HeLa and COS7 cells transfected with IL-2Rβ, and no p64 precipitation was seen even in those transfectants with both IL-2Rα and β cDNAs, which bind to IL-2 with high affinity but are not able to transduce intracellular signals. These results suggest the possibility that p64 associates with IL-2Rβ and has an important role in formation of the functional IL-2R complex.

The IL-2R exists in three different isoforms: high affinity (dissociation constant, $K_d = 10^{-11}$ M), intermediate affinity ($K_d = 10^{-9}$ M), and low affinity ($K_d = 10^{-8}$ M), consisting of a complex of α- and β-chains, the β-chain, or the α-chain, respectively (1). Transfection studies with IL-2Rα- and β-chain cDNAs have shown directly the biologic significance of each chain. The α-chain, which contains only 13 amino acid residues in its cytoplasmic domain (2, 3), has a low affinity for IL-2 binding but lacks the ability to transduce intracellular signals mediated by IL-2 (4, 5). The β-chain, which contains cytoplasmic 286 amino acid residues (6), has an intermediate affinity for IL-2 binding and transduces intracellular signals when it is expressed on lymphoid cells (7) and oligodendroglioma cells (8). However, the β-chain is not functional when it is expressed on fibroblast cells (6, 9, 10). These findings suggest two possibilities: 1) there is a cell type-specific modification of the β-chain; or 2) there are cell type-specific components associated with the β-chain which result in IL-2 binding (6). The latter possibility has been supported by recent studies using rIL-2 molecules with substitution at residue Gln-141 (11) and in vivo stimulated NK cells (12). Also, chemical cross-linking experiments with ^{125}I-labeled IL-2 have revealed the presence of at least three IL-2-cross-linked molecules, with approximate 90-, 85-, and 70-kDa molecular masses, on cells expressing the high affinity IL-2R (13-15). On the basis of their molecular masses, the 90- and 70-kDa molecules most likely represent the β- and α-chains bound with ^{125}I-IL-2, respectively, but the nature of 85-kDa molecule was obscure. We have recently demonstrated the existence of a novel component, p64, of the high affinity IL-2R, which, like the 85-kDa molecule, occurred between the α- and β-chains in PAGE (16). Because p64 is detectable by co-precipitation with the β-chain in the presence of IL-2 without any chemical cross-linker in lymphoid cells bearing high affinity IL-2R and is different from the α- and β-chains regarding protease sensitivity, p64 represents a novel molecule associated with the β chain. Therefore, we considered the possibility that p64 contributes to the formation of the functional intermediate affinity IL-2R. We present here quantitative correlations, suggesting a possible role for p64, between p64 associated with IL-2Rβ and IL-2 binding sites on cells transfected with IL-2Rβ cDNA.

Received for publication October 7, 1991.
Accepted for publication January 10, 1992.
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[1] This work was supported in part by the Grant-in-Aid for General Scientific Research and Cancer Research from the Ministry of Education, Science and Culture, and by grants from Special Coordination Founds of the Science and Technology Agency of the Japanese Government, and from the Takeda Scientific Foundation.
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MATERIALS AND METHODS

Cell lines and mAb. The cell lines used were a human T cell line, MOLT4, a human epithelial cell line, HeLa, and a simian fibroblast cell line, COS7. MOLT4 was maintained in RPMI 1640 medium supplemented with 10% FCS, 2 mM L-glutamine, and antibiotics. HeLa and COS7 were maintained in DMEM supplemented with 5% calf serum and antibiotics. The IgG1 mAb used were TU27 (17) and TU11 (18), both specific for human IL-2Rβ, and TU11, not competitive for IL-2 binding to IL-2Rβ.

Transfection of genes. Transfection of genes for IL-2Rα and IL-2Rβ into various cells was performed with electroporation (19). In brief, the *EcoRI-HindIII* fragment of human IL-2Rα cDNA (generous gift from Dr. Engels (20)) was inserted into expression vector pcDSRα by using an *XbaI* linker (21, 22) to yield pSRA4. The *XbaI* fragment

of human IL-2Rβ cDNA was inserted into pcDSRα by using an *Xba*I linker to yield pSRB5. MOLT4, HeLa, and COS7 cells were transfected with pSRB5, and HeLa and COS7 cells were transfected with pSRB5 and pSRA4 together with neomycin-resistance gene by electroporation. These transfectants were selected in the medium containing G418 (GIBCO Laboratories, Grand Island, NY), and their single-cell clones were isolated in limiting dilution cultures.

Binding assay of IL-2 and TU27 mAb. Human IL-2 (obtained from Shionogi Pharmaceutical Co., Osaka, Japan) and TU27 mAb were radiolabeled with $Na^{125}I$ (ICN Biomedicals Inc., Costa Mesa, CA) by the chloramine-T method, and the binding assays of ^{125}I-IL-2 and ^{125}I-TU27 mAb to various cells were carried out as reported previously (17). In brief, 1×10^6 cells were untreated or treated with 3 μM IL-2 or 50 μg/ml TU27 mAb for 1 h at 4°C and incubated with ^{125}I-IL-2 (1.5×10^6 dpm/pmol) or ^{125}I-TU27 mAb (4.4×10^6 dpm/pmol) for 1.5 h at 4°C. In TU27 mAb binding assays, cells were pretreated with 50 μg/ml human IgG for 1 h at 4°C. Radioactivity in the supernatants and cell pellets was measured separately, and bound/unbound fractions were analyzed by Scatchard plots.

Radioimmunoprecipitation. Radioimmunoprecipitation analyses were performed by two-dimensional PAGE as described previously (23). Briefly, $2 \sim 4 \times 10^7$ cells were radiolabeled with $Na^{125}I$ by using iodination reagent (IODO-GEN; Pierce Chemical Co., Rockford, IL). The radiolabeled cells were treated with various doses of IL-2 at 4°C for 1 h in RPMI 1640 medium containing 2% FCS and then solubilized in 2 ml of lysis buffer (25 mM Tris-HCl (pH 7.5), 140 mM NaCl, 1 mM EDTA, 2 mM PMSF, 0.1% aprotinin, and 0.5% Nonidet P-40). The lysates were incubated with TU11 mAb and protein A-Sepharose (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) pretreated with anti-mouse IgG for 4 h at 4°C. The immunoprecipitates were analysed by two-dimensional PAGE.

Peptide mapping. Peptide mapping was performed as described previously (16). In brief, gel pieces corresponding to IL-2Rβ and p64 were excised from gel of two-dimensional PAGE and put into wells of PAGE stacking gel. The wells were overlaid with 10 μl of buffer (0.125 M Tris-HCl (pH 6.8), 0.1% SDS, 1 mM EDTA, 20% glycerol, and 0.01% bromphenol blue) containing 100 μg/ml V8 protease (ICN Biomedicals). The samples were electrophoresed in the normal manner except that the current was turned off for 1 h when the bromphenol blue neared the bottom of the stacking gel. The running gel consisted of 15% polyacrylamide containing SDS.

RESULTS

A human T cell line, MOLT4, was transfected with IL-2Rβ cDNA expression plasmids, and a stable transfectant clone, MOLTβ-1, was prepared. MOLTβ-1 cells were recloned, and two subclones, MOLTβ-11 and MOLTβ-12, were obtained. These two transfectant clones were examined for the ability to bind ^{125}I-TU27 mAb and ^{125}I-IL-2 by Scatchard plots (Fig. 1). As calculated in Figure 1, MOLTβ-11 and MOLTβ-12 cells had similar TU27 mAb binding sites, 17,700 (SE = ±1600) and 18,000 (SE = ± 1300) sites/cell, respectively. However, MOLTβ-11 and MOLTβ-12 cells had significantly different numbers of IL-2 binding sites, 3700 (SE = ±320) and 6100 (SE = ± 600), respectively, per cell, although they showed the same intermediate affinity (K_d = 1.7 nM) for IL-2 binding. The ratio of IL-2 binding sites on MOLTβ-11 compared with MOLTβ-12 was 0.61. These results indicate that the number of IL-2Rβ molecules does not correlate with the number of IL-2 binding sites, suggesting the requirement for another receptor component for IL-2 binding.

MOLTβ-11 and MOLTβ-12 cells were then examined for the association of p64 with IL-2Rβ. The p64 was detectable by precipitation with TU11 mAb (specific for IL-2Rβ) in the presence of IL-2 (16). Cells, surface labeled with $Na^{125}I$, were treated with or without IL-2, and their lysates were immunoprecipitated with TU11 mAb. The immunoprecipitates were analyzed on two-dimensional PAGE (Fig. 2). TU11 mAb specifically precipitated only IL-2Rβ (molecular weight of 69,000 to 81,000, pI 4.4 to 4.7) in the lysates of cells not treated with IL-2. But it precipitated both IL-2Rβ and also p64 (molecular weight

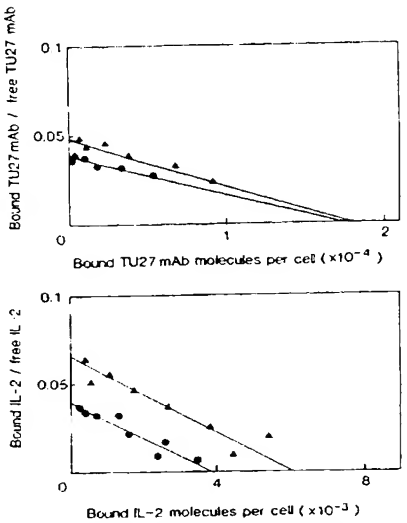

Figure 1. Scatchard plot analyses for bindings of ^{125}I-TU27 mAb and ^{125}I-IL-2. Binding assays of ^{125}I-TU27 mAb (*upper panel*) and ^{125}I-IL-2 (*lower panel*) were carried out for MOLTβ-11 (●) and MOLTβ-12 (▲), and their bindings were analyzed by Scatchard plots.

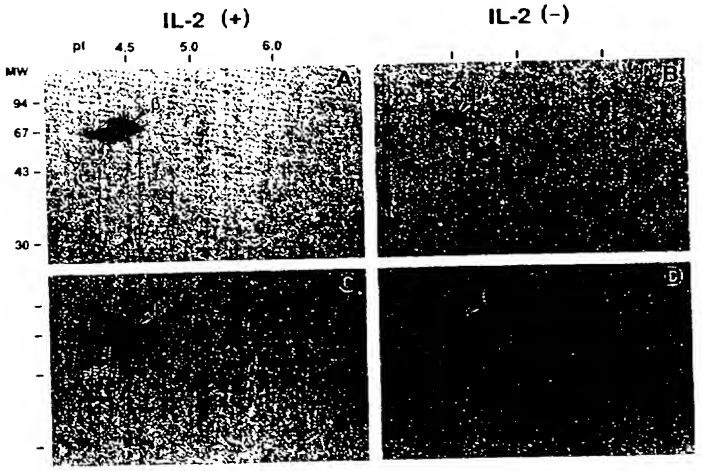

Figure 2. Detection of p64 associated with IL-2Rβ in MOLTβ-11 and MOLTβ-12 cells. MOLTβ-11 (*A* and *B*) and MOLTβ-12 (*C* and *D*) cells were surface labeled with $Na^{125}I$, treated with 30 nM IL-2 (*A* and *C*) or no IL-2 (*B* and *D*), and then lysed. Their lysates were immunoprecipitated with TU11 mAb, and the immunoprecipitates were analyzed by two-dimensional PAGE. Molecular sizes (molecular weight × 10^{-3}) and isoelectric points (pI) of standards are indicated at the *left* and the *top*, respectively.

of 61,000 to 71,000, pI 4.2 to 4.6) in those treated with IL-2. The peptide mapping of p64 molecules indicated that p64 molecules are distinct from IL-2Rβ molecules (Fig. 3). The density of p64, co-precipitated with IL-2Rβ, increased in proportion to IL-2 concentration and reached a plateau level at 10 nM IL-2 in both MOLTβ-11 and MOLTβ-12 clones whereas the density of IL-2Rβ precipitated was little changed by the IL-2 treatment (Fig. 4). The 1.7 nM IL-2, which gave a half-maximal density of p64 in both the cell clones, gave the same K_d value for IL-2R as calculated in Figure 1. The ratio of the maximum p64 densities of MOLTβ-11/MOLTβ-12 was 0.62, which is similar to the 0.61 the ratio of IL-2 binding sites (MOLTβ-11/MOLTβ-12) as noted above. Thus, the association of p64 with IL-2Rβ correlates well with the IL-2 binding ability of IL-2Rβ.

Next, because fibroblast transfectants with IL-2Rβ

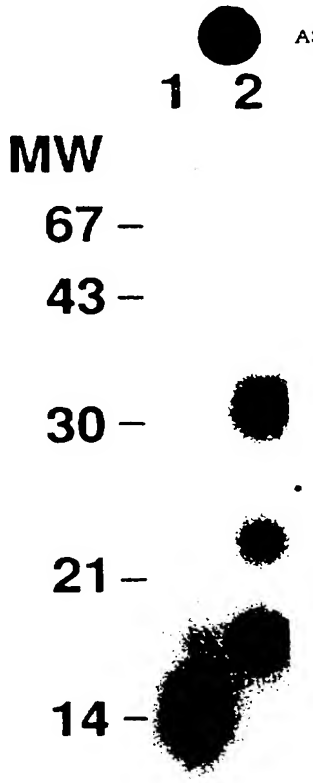

Figure 3. Peptide maps of p64 and IL-2Rβ in MOLTβ-11. IL-2Rβ (*lane 1*) and p64 (*lane 2*) were excised from the gel of MOLTβ-11 in Figure 1 and then digested with V8 protease as described under *Materials and Methods*.

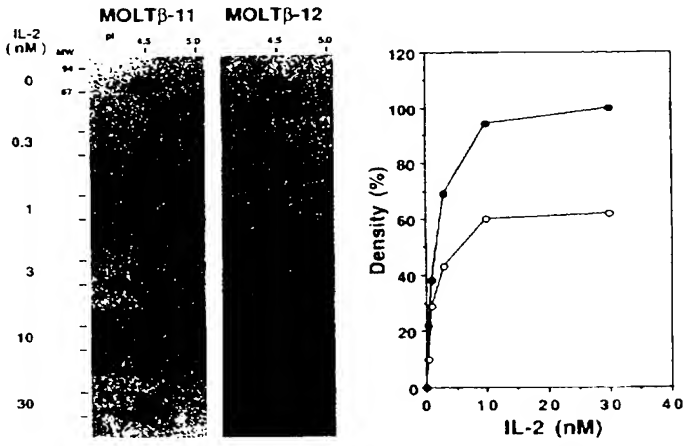

Figure 4. IL-2 dose response of p64 co-precipitation with IL-2Rβ. ^{125}I surface-labeled MOLTβ-11 and MOLTβ-12 cells were treated with the indicated concentrations of IL-2, and their lysates were immunoprecipitated with TU11 mAb. The immunoprecipitates were analyzed by two-dimensional PAGE (*left panel*). The densities of p64 spots of MOLTβ-11 (○) and MOLTβ-12 (●) were quantitated with Bio-Image Analyzer BAS-2000 (Fuji Photo Film Co., Japan), and 100% indicated the densities of the p64 spots at 30 nM IL-2 in MOLTβ-12 (*right panel*).

cDNA were shown to express IL-2Rβ molecules which have no significant affinity for IL-2 (6, 9, 10), we examined nonlymphoid transfectants for IL-2 binding. Human epithelial HeLa and simian fibroblast COS7 cell lines were transfected with IL-2Rα- and/or β-chain cDNA, and stable transfectants were obtained: HeLaβ-1 and COSβ-2, which expressed IL-2Rβ alone; and HeLaαβ-7 and COSαβ-5, which expressed both IL-2Rα and β. These transfectants were then examined for IL-2 binding ability and p64 association with IL-2Rβ. Neither IL-2 binding ability nor p64 associated with IL-2Rβ was detected in HeLaβ-1 or COSβ-2 cells (data not shown). HeLaαβ-7 cells expressed 430 sites/cell of IL-2R (K_d = 260 pM), and COSαβ-5 cells expressed 6700 sites/cell of IL-2R (K_d = 550 pM). The IL-2 binding affinities of HeLaαβ-7 and COSαβ-5 cells were significantly lower than those of high affinity IL-2R (K_d = ~10 pM) expressed on lymphoid cells (1). No p64 was detected in HeLaαβ-7 or COSαβ-5 cells treated with IL-2 (Fig. 5). In HeLaαβ-7 cells treated with IL-2, TU11mAb precipitated a small but significant amount of p55 (molecular weight of 52,000 to 59,000, pI 4.2 to 4.4) along with IL-2Rβ. Preabsorption with anti-IL-2Rα mAb diminished p55, indicating that p55 detected in HeLaαβ-7 cells is IL-2Rα (data not shown).

DISCUSSION

Previous reports on IL-2Rβ cDNA transfection revealed that the expression of IL-2Rβ induced the intermediate affinity IL-2R in lymphoid cell lines but not in fibroblast cell lines (6, 9, 10). The present study confirms these observations and further shows that, similar to the fibroblast cells, IL-2Rβ expressed on human epithelial HeLa cells has no significant affinity for IL-2 binding. The inability of IL-2Rβ expressed on nonlymphoid cell lines to bind IL-2 has been explained by either lymphoid-specific modification of IL-2Rβ or lymphoid-specific component(s) that are required for IL-2Rβ binding of IL-2. Our previous observation that the number of IL-2Rβ molecules expressed on in vivo activated NK cells does not precisely reflect the number of IL-2 binding sites on their

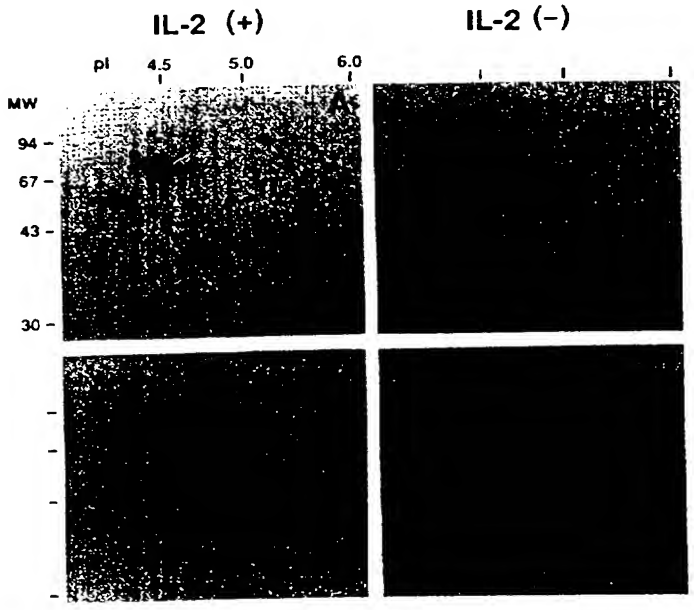

Figure 5. Immunoprecipitation of HeLaαβ-7 and COSαβ-5 cell lysate with TU11 mAb. HeLaαβ-7 (*A* and *B*) and COSαβ-5 (*C* and *D*) cells were surface labeled with Na^{125}I, treated with 30 nM IL-2 (*A* and *C*) or no IL-2 (*B* and *D*), and then lysed. Their lysates were precipitated with TU11 mAb, and the immunoprecipitates were analysed by two-dimensional PAGE.

surfaces supports the latter possibility (12). That possibility is also supported by observations that IL-2 molecules with an amino acid substitution are able to bind the high affinity IL-2R composed of the α- and β-chains but are unable to bind to IL-2R composed of the β-chain alone on lymphoid cells (11). In addition, solubilized IL-2Rβ molecules, which are produced by lymphoid cells, show much lower affinities for IL-2 binding than the IL-2Rβ (K_d = ~1 nM) on lymphoid cell surface (9, 18). Furthermore, the present study demonstrated that there was no difference in the molecular weight or isoelectric point of IL-2Rβ when lymphoid and nonlymphoid transfectants were compared and that the two transfected subclones, MOLTβ-11 and MOLTβ-12, expressed an almost equal number of IL-2Rβ molecules on their surfaces but had IL-2 binding sites that differed considerably from each other with MOLTβ-11 having significantly fewer IL-2Rβ molecules. All of these results suggested the possibility that additional IL-2R component(s) endow IL-2Rβ with an intermediate affinity to IL-2.

We demonstrated previously the existence of p64, a possible third component of IL-2R (γ-chain), by co-precipitation with IL-2Rβ in the presence of IL-2 (16). The present study expands these results and shows that p64 can be detected in association with IL-2Rβ bound to IL-2 with intermediate affinity on lymphoid cells (MOLT4) transfected with IL-2Rβ but not on the nonlymphoid cell transfectants (HeLa and COS7) expressing IL-2Rα and/or β (HeLaβ-1, COSβ-2, HeLaαβ-7, and COSαβ-5). Our recent studies indicate that the IL-2Rβ-chains on the lymphoid transfectant, MOLTβ-11, can transduce intracellular signals for tyrosine phosphorylation of IL-2Rβ and for cell growth in serum-free medium whereas IL-2Rβ-chains on nonlymphoid transfectants do not transduce these signals even though HeLaαβ-7 and COSαβ-5 expressed high affinity IL-2R.[3] These observations suggest that p64 associates with IL-2Rβ on cells in which functional IL-2R is expressed by IL-2Rβ cDNA transfection. In the present experiments the quantity of p64 detected in MOLTβ-11 and MOLTβ-12 increased depending on IL-2 dose, and the half-maximum level of p64 detection was obtained at 1.7 nM IL-2, corresponding to the K_d value of IL-2 binding affinity of IL-2Rβ on these transfectant cells. Furthermore, the ratio of p64 amounts between MOLTβ-11 and MOLTβ-12 is quite similar to the ratio of IL-2 binding sites between them, demonstrating the quantitative correlation between the amount of p64 associated with IL-2Rβ and the number of IL-2 binding sites on IL-2Rβ transfectant cells. p64 itself, in the absence of IL-2Rβ, may not have a significant affinity for IL-2 because the parental MOLT4 cells, which are thought to express p64, showed no IL-2-binding activity, and furthermore, when MOLTβ cells were pretreated with TU27 mAb, which blocks IL-2 binding to IL-2Rβ (17), they did not bind IL-2 (data not shown). From these observations we propose the possibilities that p64 functions by inducing conformational changes of IL-2Rβ which are prerequisite to IL-2 binding or, alternatively, that each single molecule of p64 and IL-2Rβ has no IL-2 binding activity, but the complex consisting of p64 and IL-2Rβ binds to IL-2 at an intermediate affinity.

It is also possible that p64 may not contribute to the acquisition of IL-2 binding ability of IL-2Rβ but that it specifically combines with IL-2Rβ bound to IL-2. In this respect our previous finding that a certain tyrosine kinase molecule associates with IL-2Rβ deserves attention (24). As mentioned above we have recently obtained evidence that tyrosine residues of IL-2Rβ can be phosphorylated by IL-2 stimulation of the MOLTβ-11 transfectant but not of the nonlymphoid transfectants. The tyrosine kinase activation by IL-2 showed the same lymphoid tropism as the p64 detection did, suggesting that p64 may be a tyrosine kinase molecule or may be implicated in tyrosine kinase activation which is essential for signal transduction from IL-2Rβ. However, it is unknown whether the detection of p64, preferentially in lymphoid cells, is regulated by the cell type-specific expression of p64 because p64 is detectable only in the precipitation with TU11 mAb specific for IL-2Rβ. p64 may be expressed on the lymphoid transfectants but not on the nonlymphoid transfectants, or p64 may be expressed on the nonlymphoid transfectants but may not associate with IL-2Rβ. Others have also found molecules associated with IL-2R, which included ICAM-I (p95) (25), HLA class I (p40–42) (26), p22 and p40 (27), p95–110 (28), and p100 (29), but there is no evidence that these molecules contribute to the formation of functional IL-2R complex. In our experimental system with no chemical cross-linker only the p64 molecule was detected in the complexes of both functional high affinity IL-2R and functional intermediate affinity IL-2R. Hence, further molecular and functional characterization of p64 should lead to elucidation of IL-2R complex formation as a functional unit.

Acknowledgments. We thank Dr. J. B. Smith for reviewing the manuscript and Drs. T. Beckers and J. W. Engels for providing the human IL-2Rα cDNA plasmid.

[3] Kumaki, S., H. Asao, T. Takeshita, Y. Kurahayashi, M Nakamura, T. Bekers, J. W. Engels, H. Nyunoya, K. Shimotohno, and K. Sugamura. 1992. Cell type-specific activation of a tyrosine kinase associated with IL-2RB chain: possible involvement of a tyrosine kinase distinct from $p56^{lck}$ in IL-2-induced growth signal transduction. *submitted for publication.*

REFERENCES

1. Smith, K. A. 1988. The interleukin 2 receptor. *Adv. Immunol. 42:165.*
2. Leonard, W. J., J. M. Depper, G. R. Crabtree, S. Rudikoff, J. Pumphrey, R. J. Robb, M. Krönke, P. B. Svetlik, N. J. Peffer, T. A. Waldmann, and W. C. Greene. 1984. Molecular cloning and expression of cDNAs for the human interleukin-2 receptor. *Nature 311:626.*
3. Nikaido, T., A. Shimizu, N. Ishida, H. Sabe, K. Teshigawara, M. Maeda, T. Uchiyama, J. Yodoi, and T. Honjo. 1984. Molecular cloning of cDNA encoding human interleukin-2 receptor. *Nature 311:631.*
4. Green, W. C., R. J. Robb, P. B. Svetlik, C. M. Rusk, J. M. Depper, and W. J. Leonard. 1985. Stable expression of cDNA encoding the human interleukin 2 receptor in eukaryotic cells. *J. Exp. Med. 162:363.*
5. Sabe, H., S. Kondo, A. Shimizu, Y. Tagaya, J. Yodoi, N. Kobayashi, M. Hatanaka, N. Matsunami, M. Maeda, T. Noma, and T. Honjo. 1986. Properties of human interleukin-2 receptors expressed on nonlymphoid cells by cDNA transfection. *Mol. Biol. Med. 2:379.*
6. Hatakeyama, M., M. Tsudo, S. Minamoto, T. Kono, T. Doi, T. Miyata, M. Miyasaka, and T. Taniguchi. 1989. Interleukin-2 receptor β chain gene: Generation of three receptor forms by cloned human α and β chain cDNA's. *Science 244:551.*
7. Hatakeyama, M., M. Mori, T. Doi, and T. Taniguchi. 1989. A restricted cytoplasmic region of IL-2-receptor β chain is essential for growth signal transduction but not for ligand binding and internalization. *Cell 59:837.*
8. Okamoto, Y., S. Minamoto, K. Shimizu, H. Mogami, and T. Taniguchi. 1990. Interleukin 2 receptor β chain expressed in an oligodendroglioma line binds interleukin 2 and delivers growth signal. *Proc. Natl. Acad. Sci. USA 87:6584.*
9. Tsudo, M., H. Karasuyama, F. Kitamura, T. Tanaka, S. Kubo, Y.

Yamamura, T. Tamatani, M. Hatakeyama, T. Taniguchi, and M. Miyasaka. 1990. The IL-2 receptor β-chain (p70): Ligand binding ability of the cDNA-encoding membrane and secreted forms. *J. Immunol. 145:599.*

10. **Minamoto, S., H. Mori, M. Hatakeyama, T. Kono, T. Doi, T. Ide, T. Uede, and T. Taniguchi.** 1990. Characterization of the heterodimeric complex of human IL-2 receptor α·β chains reconstituted in a mouse fibroblast cell line, L929. *J. Immunol. 145:2177.*
11. **Zurawski, S. M., J.-L. Imler, and G. Zurawski.** 1990. Partial agonist/antagonist mouse interleukin-2 proteins indicate that a third component of the receptor complex functions in signal transduction. *EMBO J. 9:3899.*
12. **Voss, S. D., R. J. Robb, G. Weil-Hillman, J. A. Hank, K. Sugamura, M. Tsudo, and P. M. Sondel.** 1990. Increased expression of the interleukin 2 (IL-2) receptor β chain (p70) on CD56⁺ natural killer cells after in vivo IL-2 therapy: p70 expression does not alone predict the level of intermediate affinity IL-2 binding. *J. Exp. Med. 172:1101.*
13. **Robb, R. J., C. M. Rusk, J. Yodoi, and W. C. Greene.** 1987. Interleukin 2 binding molecule distinct from the Tac protein: Analysis of its role information of high-affinity receptors. *Proc. Natl. Acad. Sci. USA 84:2002.*
14. **Dukovich, M., Y. Wano, L. B. Thuy, P. Katz, B. R. Cullen, J. H. Kehrl, and W. C. Greene.** 1987. A second human interleukin-2 binding protein that may be a component of high-affinity interleukin-2 receptors. *Nature 327:518.*
15. **Fujii, M., K. Sugamura, M. Nakamura, T. Ishii, and Y. Hinuma.** 1988. Selective inhibition of high- but not low-affinity interleukin 2 binding by lectins and anti-interleukin 2 receptor α antibody. *Microbiol. Immunol. 32:857.*
16. **Takeshita, T., H. Asao, J. Suzuki, and K. Sugamura.** 1990. An associated molecule, p64, with high-affinity interleukin 2 receptor. *Int. Immunol. 2:477.*
17. **Takeshita, T., Y. Goto, K. Tada, K. Nagata, H. Asao, and K. Sugamura.** 1989. Monoclonal antibody defining a molecule possibly identical to the p75 subunit of interleukin 2 receptor. *J. Exp. Med. 169:1323.*
18. **Suzuki, J., T. Takeshita, K. Ohbo, H. Asao, K. Tada, and K. Sugamura.** 1989. IL-2 receptor subunit, p75: Direct demonstration of its IL-2 binding ability by using a novel monoclonal antibody. *Int. Immunol. 1:373.*
19. **Ohtani, K., M. Nakamura, S. Saito, K. Nagata, K. Sugamura, and Y. Hinuma.** 1989. Electroporation: Application to human lymphoid cell lines for stable introduction of a transactivator gene of human T-cell leukemia virus type I. *Nucleic Acids Res. 17:1589.*
20. **Beckers, T., H. J. Hauser, D. Husken, and J. W. Engels.** 1988. Expression and characterization of a des-methionine mutant interleukin-2 receptor (Tac protein) with interleukin-2 binding affinity. *J. Biol. Chem. 263:8359.*
21. **Takebe, Y., M. Seiki, J. Fujisawa, P. Hoy, K. Yokota, K. Arai, M. Yoshida, and N. Arai.** 1988. SRα promoter: An efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. *Mol. Cell. Biol. 8:466.*
22. **Miura, S., K. Ohtani, N. Numata, M. Niki, K. Ohbo, Y. Ina, T. Gojobori, Y. Tanaka, H. Tozawa, M. Nakamura, and K. Sugamura.** 1991. Molecular cloning and characterization of a novel glycoprotein, gp34, that is specifically induced by the human T-cell leukemia virus type I transactivator p40^{tax}. *Mol. Cell. Biol. 11:1306.*
23. **Ishii, T., M. Kohno, M. Nakamura, Y. Hinuma, and K. Sugamura.** 1987. Characterization of interleukin 2-stimulated phosphorylation of 67 and 63 kDa protein in human T-cells. *Biochem. J. 242:211.*
24. **Asao, H., T. Takeshita, M. Nakamura, K. Nagata, and K. Sugamura.** 1990. Interleukin 2 (IL-2)-induced tyrosine phosphorylation of IL-2 receptor p75. *J. Exp. Med. 171:637.*
25. **Szollosi, J., S. Damjanovich, C. K. Goldman, M. J. Fulwyler, A. A. Aszalos, G. Goldstein, P. Rao, M. A. Talle, and T. A. Waldmann.** 1987. Flow cytometric resonance energy transfer measurements support the association of a 95-kDa peptide termed T27 with the 55-kDa Tac peptide. *Proc. Natl. Acad. Sci. USA 84:7246.*
26. **Sharon, M., J. R. Gnarra, M. Baniyash, and W. J. Leonard.** 1988. Possible association between IL-2 receptors and class I HLA molecules on T cells. *J. Immunol. 141:3512.*
27. **Saragovi, H., and T. R. Malek.** 1990. Evidence for additional subunits associated to the mouse interleukin 2 receptor p55/p75 complex. *Proc. Natl. Acad. Sci. USA 87:11.*
28. **Colamonici, O. R., L. M. Neckers, and A. Rosolen.** 1990. Putative γ-subunit of the IL-2 receptor is detected in low, intermediate, and high affinity IL-2 receptor-bearing cells. *J. Immunol. 145:155.*
29. **Sharon, M., J. R. Gnarra, and W. J. Leonard.** 1990. A 100-kilodalton protein is associated with the murine interleukin 2 receptor: Biochemical evidence that p100 is distinct from the α and β chains. *Proc. Natl. Acad. Sci. USA 87:4869.*

REVIEW

Multichain Interleukin-2 Receptor: A Target for Immunotherapy in Lymphoma

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Activation of resting T cells induces synthesis of interleukin-2 (IL-2) and expression of its specific high-affinity receptor. We proposed a multichain model for the high-affinity IL-2 receptor in which both a 55-kilodalton IL-2-binding peptide identified by the anti-Tac monoclonal antibody and a 70/75-kilodalton IL-2-binding peptide are associated in a receptor complex. The IL-2 receptor is proving to be an extraordinarily versatile therapeutic target, since it is expressed by the abnormal T cells in patients with certain lymphoid malignancies or autoimmune disorders and in individuals rejecting allografts, whereas it is not expressed by normal resting cells. Monoclonal antibodies and toxin–lymphokine conjugates directed toward IL-2 receptors represent novel therapeutic agents for these clinical conditions. [J Natl Cancer Inst 81:914–923, 1989]

The use of chemotherapeutic agents has resulted in the cure of some types of human cancer. However, many types of cancer either initially are unresponsive or subsequently acquire resistance to chemotherapy. The hybridoma technique of Kohler and Milstein (*1*) has rekindled interest in the use of antibodies as therapeutic agents in the treatment of cancer patients (*2–4*). Many in vitro studies have shown selective high-affinity binding of monoclonal antibodies to tumor cells. Furthermore, certain unconjugated murine monoclonal antibodies have had antitumor activity against human tumor xenografts in nude mice (*5*) and have, in certain cases, been curative in animal tumor models (*6*). Monoclonal antibodies directed against tumor-associated antigens have been used since 1981 in at least 25 clinical trials that have involved 13 unconjugated murine monoclonal antibodies. Twenty-three partial and three complete remissions were reported in the 185 patients included in these studies (*3*). In the remaining 86% of the cases, however, there were no beneficial clinical responses. There have been a number of explanations for the low therapeutic efficacy observed. One of the primary factors is that many of the monoclonal antibodies employed are not cytocidal or cytostatic against human neoplastic cells. Furthermore, in most cases, the antibodies were not directed against a vital structure present on the surface of malignant cells, such as a receptor for a growth factor required for tumor cell proliferation.

Recent studies on the interleukin-2 (IL-2)–IL-2 receptor system have provided the scientific basis for specific immune intervention strategies. The clinical applications of anti-IL-2 receptor-directed therapy provide a new perspective for the treatment of certain lymphoid neoplasias and autoimmune disorders and for the prevention of allograft rejection.

The IL-2 receptor-directed therapy depends on a series of observations. Successful T-cell-mediated immune responses require that the T cells change from a resting to an activated state. The activation of T cells requires two sets of signals from cell surface receptors to the nucleus. The first signal is initiated when appropriately processed and presented foreign antigen interacts with the 90-kilodalton polymorphic heterodimeric T-cell surface receptor for the specific antigen. After the interaction of antigen, presented in the context of products of the major histocompatibility complex (MHC) and interleukin-1 or interleukin-6, with the antigen receptor, T cells synthesize IL-2 (*7,8*). To exert its biologic effect, IL-2 must interact with specific high-affinity membrane receptors. Resting T cells do not express high-affinity IL-2 receptors; however, after activation with an antigen or mitogen, T cells rapidly express receptors (*9–11*). Thus, the growth factor IL-2 and the high-affinity form of its receptor are absent in resting T cells, but after activation both proteins are expressed. Although the interaction of appropriately presented antigen with its specific receptor confers specificity for a given immune response, the interaction of IL-2 with high-affinity IL-2 receptors determines its magnitude and duration.

Using purified, biosynthetically labeled IL-2, Robb et al. (*9*) demonstrated specific, saturable, high-affinity binding

Received April 27, 1989; accepted May 9, 1989.

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sites on IL-2-dependent T-cell lines as well as on mitogen- and alloantigen-activated T cells. Further progress in the analysis of the structure, function, and expression of the human IL-2 receptor was greatly facilitated by our production (*12*) of an IgG_{2a} mouse monoclonal antibody (termed "anti-Tac") that is now known to recognize the human IL-2 receptor (*13,14*). We have utilized the anti-Tac monoclonal antibody and radiolabeled IL-2 in cross-linking studies for the following reasons: (*a*) to define multiple IL-2-binding peptides that participate in the human receptor for IL-2; (*b*) to molecularly clone complementary DNAs (cDNAs) for the 55-kilodalton peptide of the human IL-2 receptor; (*c*) to define the cellular distribution of IL-2 receptors; (*d*) to determine the immunoregulatory effects that require the interaction of IL-2 with its receptor; and (*e*) to analyze disorders of IL-2 receptor expression on leukemia cells as well as those T cells participating in select autoimmune disorders. To exploit the fact that IL-2 receptors are present on abnormally activated T cells but not on normal resting cells, clinical trials of different forms of IL-2 receptor-directed therapy have been initiated. These trials involve patients with neoplastic or autoimmune disorders as well as patients receiving organ allografts.

Chemical Characterization of Multichain IL-2 Receptor

The IL-2-binding receptor peptide identified by the anti-Tac monoclonal antibody on phytohemagglutinin (PHA)-activated normal lymphocytes is a 55-kilodalton glycoprotein (*13,14*) that is sulfated (*15*) and phosphorylated on a serine residue (*16*). A series of issues were difficult to resolve when only the 55-kilodalton Tac peptide was considered. Specifically, initial receptor binding studies with radiolabeled IL-2 and anti-Tac suggested that activated normal T-cell populations and leukemic T-cell populations express fivefold to 20-fold more binding sites for anti-Tac than for IL-2; that is, although only 4,000 high-affinity (10^{-11} *M*) IL-2 receptors were detected on PHA-activated lymphocytes in binding assays performed with radiolabeled IL-2, 30,000-60,000 sites were demonstrable with anti-Tac (*17*). However, Robb et al. (*18*) repeated these studies but utilized greater quantities of IL-2 than had been used previously. They showed that, in terms of affinity, there are two classes of IL-2 receptors. In general, most cell populations display 5%–20% of their IL-2 receptors with an apparent affinity of 10^{-11} *M*, whereas the remaining receptors bind IL-2 with an affinity three logs lower (10^{-8} *M*).

The Tac peptide was shown to participate in both the high- and the low-affinity forms of the IL-2 receptor. Isolation of cDNAs encoding the Tac peptide did not provide an explanation for the great difference in affinity between high- and low-affinity receptors. In studies of this issue, Tac peptide cDNA was shown to reconstitute high-affinity receptors when it was transfected into lymphoid cells but not when it was transfected into nonlymphoid cells (*19–21*). Furthermore, low-affinity receptors were converted to the high-affinity form after membranes from human T cells were fused with membranes from L cells transfected with cDNA encoding the p55 murine counterpart of the Tac peptide (*22*). These two observations supported the view that a cofactor or "converter" protein from T cells somehow imparted high affinity to the low-affinity binding protein (*19–23*). However, no one considered it likely that a separate IL-2-binding peptide might account for the observations.

Certain additional observations led us to conduct experiments that helped resolve this issue. IL-2 was shown to up-regulate the expression of the Tac peptide in multiple cell types, including some cells that did not initially express the Tac peptide (*24,25*). Furthermore, it had been shown that certain cells not expressing Tac, including large granular lymphocytes (LGLs), which are precursors of natural killer (NK) cells, and lymphokine-activated killer (LAK) cells, could be activated to become efficient killers by IL-2 (*26,27*). Finally, the cell line MLA-144 was Tac negative yet manifested 4,000 IL-2-binding sites with intermediate affinity. These observations led us to consider the possibility that the high-affinity IL-2 receptor was not a single peptide but rather a receptor complex that included the Tac peptide as well as novel non-Tac peptides. In studies initially presented at the Sixth International Immunology Congress in July 1986, we utilized radiolabeled IL-2 in cross-linking to define the size of the IL-2 receptor peptides on various cell lines, including MLA-144 (*28–31*). In these studies, we employed ^{125}I-labeled IL-2 that had been cross-linked using the bifunctional agent disuccinimidyl suberate. We identified a 70/75-kilodalton IL-2-binding protein on the MLA-144 cell line. The binding of IL-2 to this peptide was blocked by excess unlabeled IL-2 but not by the anti-Tac antibody, confirming the presence of a novel 70/75-kilodalton IL-2-binding protein (p75). When a variety of T-cell lines were examined for IL-2 binding and were subjected to IL-2 cross-linking studies, we demonstrated a correlation between the affinity of IL-2 binding and the IL-2-binding peptides expressed (*30*). In these studies, cell lines bearing either the p55 Tac or the p75 peptide alone manifested low- or intermediate-affinity IL-2 binding, whereas cell lines bearing both peptides manifested both high- and low-affinity receptors. In light of these observations, we proposed a multichain model for the high-affinity IL-2 receptor. In this model an independently existing p55 or p75 peptide would create low- or intermediate-affinity receptors, whereas high-affinity receptors would be created when both receptors were expressed and noncovalently associated in a receptor complex (*28–31*). We have shown that fusion of membranes from a low-affinity IL-2 receptor cell line bearing the Tac peptide alone (MT-1) with membranes from a line with intermediate-affinity receptors bearing the p75 peptide alone (MLA-144) generated hybrid membranes bearing high-affinity receptors (*30*). These findings support the proposed multichain model for the high-affinity receptor.

In independent studies, Sharon et al. (*32*) proposed a similar model. This group used similar cross-linking studies with radiolabeled IL-2 on activated and human T-cell leukemia-lymphoma virus type I (HTLV-I)-infected T-cell lines. They identified bands at 68–72 kilodaltons and 85–92 kilodaltons. The latter band was not present when IL-2 was cross-linked to cells transfected with the Tac peptide

gene. In subsequent studies, these investigators partially purified the p70/75 peptide. Partial proteolysis with V8 protease followed by analysis of the fragments by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) yielded different peptide maps with the p55 and p70/75 complexes. These studies demonstrated a novel IL-2-binding peptide, p70/75 (also termed the "β chain"), and its association with the p55 Tac peptide (the α chain) in the high-affinity receptors. The results from these studies were confirmed and extended in subsequent studies by others (*33–35*).

Dukovich et al. (*33*) identified the p70/75 IL-2-binding peptide in the absence of the Tac peptide on the surface of a B-cell line, SKW6.4B, that secretes immunoglobulin in response to IL-2. In addition, Robb et al. (*34*) demonstrated that the subclones of the human leukemic NK cell line YT, which do not express the Tac antigen yet express intermediate-affinity IL-2 receptors, express the p70/75 IL-2-binding polypeptide. Furthermore, both groups of investigators showed that YT cells induced to display high-affinity receptors by various treatments, including the addition of forskolin, concomitantly express both p55 and p70/75. Moreover, Dukovich et al. (*33*) showed that the introduction of cDNA encoding p55 into MLA-144 cells by protoplast fusion led to the generation of high-affinity IL-2 receptors (K_d of 35–70 pM) in a proportion of these cells. These reconstituted, high-affinity receptors included the Tac antigen, since the addition of anti-Tac blocked the high-affinity but not the intermediate-affinity component of IL-2 binding.

Kinetic binding studies with IL-2 have provided an interesting perspective on how the two separate IL-2-binding proteins cooperate to form the high-affinity receptor. Each chain reacts very differently with IL-2, with distinct kinetic and equilibrium binding constants. The on-and-off rate for IL-2 binding to the Tac protein is rapid (5–10 sec), while the on-and-off rate for IL-2 binding to the p70/75 protein is markedly slower (>40 min) (*35–37*). The kinetic binding data obtained when high-affinity receptors are analyzed show that the association rate of this receptor depends on the fast-reacting p55 Tac chain, whereas the dissociation rate is derived from the slow-reacting p75 chain. Because the affinity of binding at equilibrium is determined by the ratio of the dissociation constant and the association rate constant, this kinetic cooperation between the low- and intermediate-affinity ligand-binding sites results in a receptor with a high affinity for IL-2 (*35–37*).

Evidence suggests a more complex subunit structure that involves peptides in addition to the p55 and p75 IL-2-binding peptides. In the initial biochemical characterization of the human p55 subunit using anti-Tac, bands of relative molecular mass (M_r) 113,000 and 180,000 were obtained (*13,14*). Subsequently, molecular species with M_r 35,000, 44,000, 116,000, 135,000, and 180,000 were identified with anti-p55 precipitates from ^{32}P labeled IL-2-stimulated human T cells (*38–40*). Similar molecular species of p40, p100, p135, and p180 were identified in activated murine T cells and lines (*41*). Furthermore, the addition of IL-2 to concanavalin A-activated splenic T cells induced the rapid and time-dependent phosphorylation of membrane proteins with M_r 34,000, 40,000, 55,000–58,000, 66,000, 90,000, 105,000 and 115,000 (*39*). Although class I MHC molecules have been cross-linked to radiolabeled IL-2, the p40 species has been identified to be a novel peptide other than a class I MHC molecule or actin. In studies directed toward identification of activation antigens on T cells, two monoclonal antibodies, OKT27 and OKT27b, were produced that react with distinct epitopes of a 95-kilodalton peptide. The OKT27b antibody inconsistently coprecipitated the 55-kilodalton Tac peptide as well as the 95-kilodalton peptide (*42*). A flow cytometric energy-transfer technique was used to demonstrate a close nonrandom proximity between the p55 Tac and the 95-kilodalton T27 peptides (*42*). Furthermore, fluorescence-photobleaching recovery measurements suggest that the Tac and T27 peptides interact physically in situ in HUT-102 membranes (*43*). In independent chemical cross-linking studies with radiolabeled IL-2, Herrmann and Diamantstein (*44*), Saragovi and Malek (*45*), and Sharon et al. (*46*) presented evidence for a 95- to 105-kilodalton IL-2-binding peptide in mice associated with the 55- and 75-kilodalton chains of the high-affinity form of the IL-2 receptor on mouse T-cell blasts, CTLL-16 cells, and sublines of EL-4 transfected with the gene encoding the p55 peptide. This 95- to 105-kilodalton peptide was not precipitated by an anti-p55-specific antibody. Taken together, these studies suggest that a p95–105 peptide is associated with the p55 and p75 peptides in the multisubunit high-affinity IL-2 receptor.

Recently, Saragovi and Malek (*41*) provided evidence indicating that the p75 IL-2-binding peptide of mice exists as a p75/p22–30 heterodimer. The p75 peptide, precipitated by immobilized IL-2 or by coprecipitation with anti-p55 antibody immunoprecipitations, yielded an M_r-105,000 species when analyzed under nonreducing conditions. Under reducing conditions and in diagonal nonreduced versus reduced SDS-PAGE analysis, 75- and 22-kilodalton species were identified from the appropriately internally radiolabeled cells.

Finally, evidence suggests that there are two independent M_r-70,000 and M_r-75,000 peptides in the high-affinity receptor. In radiolabeled IL-2 cross-linking studies, two bands are usually observed in the 70,000/75,000 range that could theoretically represent two protein species or differential glycosylation of a single species (*47,48*). Furthermore, although a series of antibodies have been generated that completely inhibit IL-2 binding to the p75 IL-2-binding peptide, none has agonist activity for activated T cells. In contrast, other monoclonal antibodies have been generated that recognize a 70/75-kilodalton peptide on human activated T cells and LGLs that have agonist activity. However, these monoclonal antibodies do not fulfill the criteria for an antibody to an IL-2-binding protein. The most extensively studied monoclonal antibodies of this group, YTA-1 and YTA-2 described by Nakamura et al. (*49*), recognize 75-kilodalton molecules on human LGLs (*49*). However, these antibodies do not block IL-2 binding to p75 IL-2 receptors expressed on LGLs, and they do not precipitate IL-2 cross-linked to the prototypical p75 IL-2-binding peptide. Nevertheless, these monoclonal antibodies down-regulate/modulate

high-affinity IL-2 receptors from peripheral blood mononuclear cells in 24-hour cultures. Furthermore, YTA-1 and YTA-2 were mitogenic and were different from other mitogenic monoclonal antibodies, such as CD3, CD2, and CD28. Clearly, the p75 peptide identified by monoclonal antibodies such as YTA-1 may not be a participant in the high-affinity IL-2 receptor. Alternatively, this peptide may be a non-IL-2-binding peptide that plays a role in the transduction of the signal from the two IL-2-binding subunits p55 and p75.

In summary, with the use of coprecipitation analysis and radiolabeled IL-2 cross-linking procedures, as well as other techniques, a series of peptides of M_r 22,000, 35,000, 40,000, 75,000, 95,000–105,000, 135,000, and 180,000 have been associated with the two IL-2-binding peptides. Further studies, including molecular cloning followed by expression of the peptides in concert in cells not expressing IL-2 receptor, will be required to define which of these peptides plays a meaningful role in the multisubunit IL-2 receptor.

Structure–Function Relationship of Interaction of IL-2 and IL-2 Receptor

The three-dimensional structure of the 133-amino-acid lymphokine IL-2 has been defined (*50*). These studies, in conjunction with studies using site-specific mutagenesis of IL-2 and monoclonal antibodies directed toward defined regions of IL-2 in neutralization and binding assays (*51,52*), have aided in the analysis of the structure–function relationships of human IL-2. Furthermore, they have led to the identification of the amino acid residues required for binding to the different IL-2 receptor peptides and for biologic activity. IL-2 has an α-helical tertiary structure involving six α helices; this structure suggests that certain portions of the molecule form a structural scaffold that underlies the receptor-binding facet of the molecule (*50*). A short helical segment (helix A, amino acid residues 11–19) and the neighboring amino acid 20 are required for biologic activity and appear to be involved in binding to the p75 IL-2-binding peptide (*51,52*). The second helix on the structural scaffold is an extended loop involving residues 33–56 that form a helix interrupted in the middle by Pro^{47}. These two segments are referred to as B and B′. These segments appear to be required for binding to the p55 Tac peptide. An additional α helix E (amino acids 107–113) is also positioned on the binding plane. However, this region of IL-2 has not been extensively studied. Finally, the carboxy-terminal residues 121–133 and two of the three cysteine residues (amino acids 58 and 105) are required for full biologic activity and binding (*51*).

Molecular Cloning of cDNAs for Human 55-Kilodalton Tac IL-2 Receptor Peptide

Three laboratories (*53–55*) have succeeded in cloning cDNAs for the p55 Tac IL-2 receptor peptide. The deduced amino acid sequence of the IL-2 receptor indicates that this peptide is composed of 251 amino acids as well as a 21-amino-acid signal peptide. The receptor contains two potential N-linked glycosylation sites and multiple possible O-linked carbohydrate sites. Finally, there is a single hydrophobic membrane region of 19 amino acids and a very short (13-amino-acid) cytoplasmic domain. Potential phosphate acceptor sites (serine and threonine, but not tyrosine) are present within the intracytoplasmic domain. However, the cytoplasmic domain of the IL-2 receptor peptide identified by anti-Tac appears to be too small for enzymatic function. This receptor differs from other known growth factor receptors that have large intracytoplasmic domains with tyrosine kinase activity. Thus, the p75 peptide or other peptides associated with the Tac peptide may play a critical role in the transduction of the IL-2 signal to the nucleus.

Distribution of IL-2 Receptors

The majority of resting T cells, B cells, or monocytes in the circulation do not display the 55-kilodalton peptide of the IL-2 receptor. Specifically, less than 5% of freshly isolated unstimulated human peripheral blood T lymphocytes react with the anti-Tac monoclonal antibody. However, most T lymphocytes can be induced to express IL-2 receptors by interaction with lectins, by monoclonal antibodies to the T-cell antigen receptor complex, or by alloantigen stimulation (*12,17*). In addition, malignant as well as activated normal B cells display the Tac antigen and manifest high-affinity receptors for IL-2 (*25,56*).

IL-2 receptors identified with the anti-Tac monoclonal antibody have been detected on activated cells of the monocyte–macrophage series, including cultured monocytes, Kupffer's cells of the liver, cultured lung macrophages, and Langerhans' cells of the skin (*57,58*).

Rubin et al. (*59*) demonstrated that activated normal peripheral blood mononuclear cells and certain lines of T- or B-cell origin release a soluble form of the IL-2 receptors into the culture medium. Using an enzyme-linked immunosorbent assay with two monoclonal antibodies that recognize distinct epitopes on the human IL-2 Tac receptor, they showed that normal individuals have measurable amounts of IL-2 receptors in their plasma and that certain lymphoreticular malignancies, autoimmune disorders, and allograft reactions are associated with elevated plasma levels of this receptor. The release of soluble IL-2 receptors appears to be a consequence of activation of various cell types that may play a role in the regulation of the immune response. Furthermore, determination of plasma levels of IL-2 receptors appears to provide a valuable noninvasive approach to the analysis of both normal and disease-associated lymphocyte activation in vivo.

With the use of specific monoclonal antibodies, the presence of the p75 IL-2-binding peptide has been demonstrated on circulating CD8 but not CD4 T cells (*60*). Furthermore, this peptide is expressed along with the 55-kilodalton Tac peptide on activated B and CD4 and CD8 lymphocytes. In addition, it is expressed on certain circulating cells that do not express the Tac antigen. LGLs not expressing Tac can be stimulated by IL-2 to enhance NK cell activity and to generate the cytotoxic LAK cells that can lyse NK cell-resistant tumor targets (*26,27*). Using cross-linking methodology with radiolabeled IL-2, we demonstrated that normal LGLs and leukemic LGLs from all individuals tested expressed the p75

IL-2-binding peptide but did not express the Tac peptide (*61*). Flow cytometric analysis with anti-p75 monoclonal antibodies confirmed that this peptide is expressed in Leu-19$^+$ NK cells (*60*).

Lymphocyte Functions Regulated by Interaction of IL-2 With Its Receptor

Extensive studies have been attempted to define the contributions to lymphocyte function made by the p55 and p75 peptides when expressed alone and when associated in the high-affinity IL-2 receptor complex. Studies using anti-Tac, as well as studies performed on cells expressing the p75 IL-2 peptide alone, clearly show that the p55 IL-2-binding peptide is not required for the transduction of signals initiated by high concentrations of IL-2. However, the p55 peptide plays a critical role in the high-affinity receptor. In contrast to the constitutive nature of p75 expression on certain lymphoid cells, the Tac gene is highly regulated. Furthermore, IL-2 binds to and dissociates rapidly from the p55 peptide, whereas it binds to and dissociates relatively slowly from the p75 chains (*35–37*). The association rate of the heterodimer is determined by the fast-reacting p55 chain. Furthermore, the affinity of the p55/p75 heterodimer is 100-fold greater ($K_d = 10^{-11}$ *M*) than that of the p75 peptide alone ($K_d = 10^{-9}$ *M*). This increase in affinity is required for a cellular response to the exceedingly low levels of IL-2 present in vivo. The importance of IL-2 interaction with the Tac peptide is underscored by the effects on lymphocyte functions observed after addition of the anti-Tac monoclonal antibody. This antibody disrupts the interaction of IL-2 with the p55 peptide and thus disrupts IL-2 interaction with the high-affinity p55/p75 receptor. The addition of anti-Tac to cultures of human peripheral blood mononuclear cells inhibited a variety of immune reactions. Anti-Tac profoundly inhibited the proliferation of T lymphocytes stimulated by soluble antigens and by cell surface antigens (e.g., autologous and allogeneic mixed lymphocyte reactions) (*62,63*). Upon activation, human T cells acquire other surface structures that in large measure are growth factor receptors that are not easy to detect during their resting stage (*64,65*). The addition of anti-Tac at the initiation of T-cell cultures stimulated by mitogens, antigens, or the T3 antibody inhibited the expression of the late-appearing activation proteins examined, the insulin and transferrin receptors, and the Ia proteins (*64,65*). Anti-Tac was also shown to inhibit a series of T-cell functions, including the generation of both cytotoxic and suppressor T lymphocytes in allogeneic cell cultures, but it did not inhibit their action once it was generated. In contrast to the action on T cells, anti-Tac did not inhibit the IL-2-induced activation of LGLs into effective NK and LAK cells (*26,27*). As noted above, LGLs express the p75 but not the 55-kilodalton peptide. Furthermore, up-regulation of the expression of Tac messenger RNA (mRNA) and Tac peptide by IL-2 has been demonstrated for a number of cell types (e.g., LGLs, B cells, and resting T cells), including some that initially express few if any Tac molecules (*24,25,66*). The addition of IL-2 to such Tac$^-$ cells, including LGL leukemia cells, augmented transcription of the Tac gene and induced the expression of the Tac peptide (*61*). Neither the IL-2-induced activation of LGLs nor the up-regulation of the Tac gene expression was inhibited by the addition of anti-Tac. These results strongly suggest that the p75 peptide is responsible for IL-2-induced activation of LGLs and that the p75 peptide can mediate an IL-2 signal without co-expression of the Tac peptide. The functional effects of the addition of antibodies that completely block the interaction of IL-2 with the p75 β chain have been assessed on LGLs that express this IL-2-binding peptide alone. As anticipated, the addition of such β-chain-specific blocking antibodies inhibited IL-2-mediated functions, including the induction of efficient NK cell and LAK cell activities and the expression of the p55 α chain. Surprisingly, the addition of such antibodies was relatively to completely ineffective in inhibiting IL-2-induced proliferation of T cells expressing high affinity (*60,67*). Possibly, the interaction of IL-2 with the p55 IL-2-binding peptide alters either the conformation of the p55 peptide or that of IL-2 itself in such a way that the monoclonal antibody to p75 is no longer effective in preventing signaling initiated by this p75 peptide. Taken as a whole, the p75 peptide appears to play an important role in the IL-2-mediated immune response not only by participating with the Tac peptide in the formation of the high-affinity receptor complex on T cells but also by contributing to the initial triggering of LGL activation so that these cells become efficient NK and LAK cells.

Disorders of IL-2 Receptor Expression in Malignant and Autoimmune Diseases

Normal resting T cells, B cells, and monocytes do not express the Tac peptide of the IL-2 receptor. In contrast, this receptor is expressed by a proportion of the abnormal cells in certain forms of lymphoid neoplasia, in select autoimmune diseases, and in individuals rejecting allografts. That is, a proportion of the abnormal cells in these diseases express the Tac antigen on their surface. Furthermore, the serum concentration of the soluble form of the Tac peptide is elevated. In terms of the neoplasias, certain T-cell, B-cell, monocytic, and even granulocytic leukemias express the Tac antigen. Specifically, virtually all of the abnormal cells of patients with HTLV-I adult T-cell leukemia express the Tac antigen (*68,69*). Similarly, a proportion of patients with cutaneous T-cell lymphomas, including the Sézary syndrome and mycosis fungoides, express the Tac peptide (*68, 70–72*). Furthermore, the malignant B cells of virtually all patients with hairy cell leukemia and of a proportion of patients with large and mixed cell diffuse lymphomas are Tac$^+$ (*56*). The Tac antigen is also expressed on the Reed-Sternberg cells of patients with Hodgkin's disease and on the malignant cells of patients with true histiocytic lymphoma (*72*). Finally, a proportion of the leukemia cells of patients with chronic or acute myelogenous leukemia are Tac$^+$. In addition to these Tac-expressing leukemias and lymphomas, there are certain leukemias (e.g., acute lymphoblastic leukemia and LGL leukemia) that do not express the Tac peptide but do express the p75 peptide of the IL-2 receptor (*73,74*).

Autoimmune diseases may also be associated with disor-

ders of Tac antigen expression. A proportion of the mononuclear cells in the involved tissues express the Tac antigen, and the serum concentration of the soluble form of the Tac peptide is elevated (*75*). Such evidence for T-cell activation and disorders of Tac antigen expression are present in patients with rheumatoid arthritis or systemic lupus erythematosus, subsets of patients with aplastic anemia, and individuals with HTLV-I-associated tropical spastic paraparesis (*75–78*). Disorders of IL-2 receptor expression have also been demonstrated in animal models of these diseases, including adjuvant arthritis of rats, the rodent models of type I diabetes (NOD mouse and BB rat), experimental allergic encephalomyelitis of mice, and rodent models of systemic lupus erythematosus (*79–81*).

Disorders of IL-2 Receptor Expression in Adult T-Cell Leukemia

A distinct form of mature T-cell leukemia was defined by Takasuki et al. (*82*) and termed "adult T-cell leukemia" (ATL). ATL is a malignant proliferation of mature T cells that tend to infiltrate the skin. Cases of ATL are associated with hypercalcemia and an immunodeficiency state that usually has a very aggressive course (*82,83*). The ATL cases are clustered within families and in geographic areas, occurring where HTLV-I is endemic in the southwest of Japan, the Caribbean basin, and sub-Saharan Africa. HTLV-I has been shown to be a primary etiologic agent in ATL (*84*). The leukemia cells are usually of the CD4-positive phenotype, yet functionally do not manifest T-cell helper activity but function as suppressors–effectors and inhibit the immunoglobulin synthesis on cocultured pokeweed mitogen-stimulated mononuclear target populations (*68*). The leukemia cells that we and others have examined from patients with HTLV-I-associated ATL expressed high- and low-affinity IL-2 receptors, including the Tac peptide (*68,69*). An analysis of HTLV-I and its protein products suggests a potential mechanism for this association between HTLV-I and constitutive IL-2 receptor expression. The complete sequence of HTLV-I has been determined by Seiki et al. (*85*). In addition to the presence of typical long terminal repeats (LTRs), gag, pol, and env genes, retroviral gene sequences common to other groups of retroviruses, HTLV-I and HTLV-II were shown to contain a genomic region between env and the 3′ LTR referred to as pX. This region encodes at least three peptides of 21, 27, and 40–42 kilodaltons. Sodroski et al. (*86*) demonstrated that one of these, a 42-kilodalton protein that they termed the "tat" (transactivator of transcription) protein (now termed "tax"), is essential for viral replication. The mRNA for this protein is produced by a double splicing event. To stimulate transcription, the tax protein requires the presence of three 21-base pair (bp) enhancer-like repeats within the LTR of HTLV-I (*87,88*).

Studies involving the transfection of cDNA encoding the tax product of HTLV-I into Jurkat T cells showed that the tax protein plays a central role in increasing the transcription of host genes governing human IL-2 and IL-2 p55 receptor peptide expression (*89–94*). In these transient expression studies, the tax protein stimulated an increase in IL-2 receptor-promoter activity. In contrast, tax alone had little effect on IL-2 promoter activity in Jurkat T cells but markedly synergized with other mitogenic stimuli (PHA or phorbol myristate acetate), which alone were ineffective. A direct interaction of the tax protein with specific DNA sequences seems unlikely, since the promoters of IL-2 and IL-2 receptor genes do not share strong sequence homologies with the 21-bp enhancer-like regions of the LTR of HTLV-I that have been shown to be involved in tax-induced *trans*-activation. Ruben et al. (*90*) demonstrated that the 12-base sequence motif in the −255 to −267 5′ region to the IL-2 receptor Tac gene is required for activation by the tax gene. This is the site of action of the nuclear factor NF-κ B. Ruben et al. suggest that the activation of the IL-2 receptor Tac gene expression by HTLV-I tax protein occurs through an interaction with, or activation of, a host transcription factor with properties similar, if not identical, to those of NF-κ B. Others used mutational analyses to suggest that the NF-κ B-binding site is not required for tax *trans*-activation. In addition, Fujii et al. (*95*) pointed out that a consensus sequence C-C(A + T-rich)-G-G is a protein-binding site shared by the IL-2 receptor and by c-fos. This region is required for c-fos gene activation by tax. As noted above, an uncontrolled autocrine T-cell growth model has been proposed for the early events of HTLV-I-induced T-cell transformation. Thus, the tax protein may play an important role in HTLV-I-induced malignancy by deregulating the expression of the cellular genes encoding IL-2 and the IL-2 receptor, which are involved in the normal control of T-cell proliferation.

IL-2 Receptor as Target for Therapy in Patients With Tac-Expressing Leukemia–Lymphoma, in Patients With Autoimmune Disorders, and in Patients Receiving Organ Allografts

The scientific basis for therapeutic trials using agents to eliminate the IL-2 receptor-expressing cells is the observation that T cells in patients with certain lymphoid malignancies, in patients with select autoimmune diseases, and in patients rejecting allografts express IL-2 receptors identified by the anti-Tac monoclonal antibody, whereas normal resting cells and their precursors do not. Such agents could theoretically eliminate Tac-expressing leukemia cells or activated T cells involved in other disease states, while retaining the Tac^- mature normal T cells and their precursors that express the full repertoire of antigen receptors for T-cell immune responses. The agents that we have used include (*a*) unmodified anti-Tac monoclonal antibody; (*b*) toxin conjugates of anti-Tac [e.g., A chain of ricin toxin, *Pseudomonas* exotoxin (PE) and truncated PE (PE40)]; (*c*) IL-2 truncated toxin fusion proteins (e.g., IL-2 PE40); (*d*) α- and β-emitting isotopic (e.g., ^{212}Bi and ^{90}Y) chelates of anti-Tac; and (*e*) hybrid "humanized" anti-Tac with mouse light- and heavy-chain variable or hypervariable regions joined to the human constant κ light-chain and IgG_1 or IgG_3 heavy-chain regions.

We have initiated a clinical trial to evaluate the efficacy of iv administered anti-Tac monoclonal antibody in the treatment of patients with ATL (*96,97*). None of the nine patients treated suffered any untoward reactions, and only one, a pa-

tient with anti-Tac-induced clinical remission, produced antibodies to the mouse immunoglobulin or to the idiotype of the anti-Tac monoclonal antibody. After anti-Tac therapy, three of the patients had a temporary mixed, partial, or complete remission lasting 1, 5, and over 8 months, respectively (*97*).

These therapeutic studies have been extended by the examination of the efficacy of toxins coupled to anti-Tac to selectively inhibit protein synthesis and viability of Tac^+ ATL lines. The addition of anti-Tac antibody coupled to PE inhibited protein synthesis by Tac-expressing HUT-102-B2 cells, but not that by the acute T-cell leukemia line MOLT 4, which does not express the Tac antigen (*98*).

The initial PE-anti-Tac conjugate was hepatotoxic when administered to patients with ATL. Functional analysis of deletion mutants of the PE structural gene has shown that domain I of the 66-kilodalton PE molecule is responsible for cell recognition, domain II for translocation of the toxin across membranes, and domain III for ADP-ribosylation of elongation factor 2, the step actually responsible for cell death (*99*). A PE molecule from which domain I has been deleted (PE40) has full ADP-ribosylating activity but extremely low self-killing activity when used alone because of the loss of the cell-recognition domain. The PE40 was produced in *Escherichia coli*, purified, and conjugated to anti-Tac. The anti-Tac PE conjugates inhibited the protein synthesis of Tac-expressing T-cell lines but not that of lines not expressing Tac.

IL-2-PE40, a chimeric protein composed of human IL-2 genetically fused to the amino terminal of the modified form of PE40, was constructed to provide an alternative (lymphokine-mediated) method of delivering PE40 to the surface of IL-2 receptor Tac^+ cells (*99,100*). The IL-2-PE40, a cytotoxic protein, was produced by fusion of a cDNA-encoding human IL-2 gene to the 5′ end of a modified PE40 gene that lacks sequences encoding the cell-recognition domain (*99,100*). The addition of IL-2-PE40 led to the inhibition of protein synthesis by the toxin moiety of IL-2-PE40 when added to human cell lines expressing either the p55 or p75 or both IL-2 receptor subunits. The receptor internalization was much more efficient when high affinity receptors composed of both units were present. IL-2-PE40 is a powerful reagent for studying IL-2 receptor interactions and for analyzing pathways of human immune response and its regulation. This chimeric protein toxin is being evaluated as an agent for IL-2 receptor-directed therapy in rodents with different forms of autoimmune disease as well as in primates receiving allografts.

The action of toxin conjugates of monoclonal antibodies depends on their ability to be internalized by the cell and released into the cytoplasm. Anti-Tac bound to IL-2 receptors on leukemia cells is internalized slowly into coated pits and then endosomic vesicles. Furthermore, the toxin conjugates do not pass easily from the endosomic vesicles to the cytosol, as required for their action on elongation factor 2. To circumvent these limitations, alternative cytotoxic reagents were developed that could be conjugated to anti-Tac and that were effective when bound to the surface of Tac-expressing cells. In one case, it was shown that ^{212}Bi, an α-emitting radionuclide conjugated to anti-Tac by use of a bifunctional chelate, was well suited for this role (*101*). Activity levels of 0.5 μCi or the equivalent of 12 rad/mL of α radiation targeted by ^{212}Bi-labeled anti-Tac eliminated more than 98% of the proliferative capacity of the HUT-102 cells, with only a modest effect on IL-2 receptor-negative lines. This specific cytotoxicity was blocked by excess unlabeled anti-Tac but not by human IgG.

In parallel studies, the β-emitting ^{90}Y was chelated to anti-Tac with the use of the chelate 1(2)-methyl-4-(P-isothiocyanatobenzyl)diethylenetriamine-pentaacetic acid, which did not permit elution of the radiolabeled yttrium from the monoclonal antibody (*102*). As noted below, rhesus monkeys receiving a xenograft of a cynomolgus monkey heart showed a marked prolongation of xenograft survival after the administration of ^{90}Y-labeled anti-Tac. Thus, ^{212}Bi-labeled anti-Tac and ^{90}Y-labeled anti-Tac are potentially effective and specific immunocytotoxic agents for the elimination of Tac-expressing cells.

In addition to their use in the therapy of patients with ATL, these IL-2 receptor-directed approaches are being evaluated for their ability to eliminate IL-2 receptor-expressing T cells in other clinical states, including certain autoimmune disorders. In these disorders, the lymphocytes infiltrating the affected organs express the Tac antigen, and the soluble form of the IL-2 receptor in the serum is elevated. Appropriate anti-IL-2 receptor antibodies were shown to suppress murine diabetic insulitis, lupus nephritis, experimental allergic encephalomyelitis, and adjuvant arthritis (*79,80,103*). With these encouraging results in animal models, IL-2 receptor–directed therapy is being initiated in patients with autoimmune disorders.

Monoclonal antibodies that recognize the IL-2 receptor have also been used to inhibit the graft-versus-host reaction and organ allograft rejection. Antibodies to the p55 IL-2 receptor (Tac) were shown to inhibit the proliferation of T cells to foreign histocompatibility antigens expressed on the donor organ and to prevent the generation of cytotoxic T cells in allogeneic cell cocultures (*62,63*). Volk et al. (*104*) demonstrated that the acute graft-versus-host reaction across a strong MHC barrier in mice can be suppressed by AMT-13, a monoclonal antibody directed against the 55-kilodalton IL-2 receptor on activated mouse lymphocytes. Furthermore, in studies by Kirkman et al. (*105*), the survival of cardiac allografts was prolonged in some cases to indefinite survival in rodent recipients treated with an anti-IL-2 receptor monoclonal antibody. In parallel studies, the administration of anti-Tac for the initial 10 days after transplantation prolonged the survival of renal allografts in cynomolgus monkeys (*106*). Furthermore, Bacha et al. (*107*) achieved prolongation of allograft survival and suppression of delayed-type hypersensitivity with genetically engineered diphtheria toxin linked to the NH_2 terminus of human IL-2. However, unmodified anti-Tac did not lead to a prolongation of graft survival in heterotopic cardiac xenografts in which rhesus monkeys received cardiac xenografts from cynomolgus donors. In contrast, animals receiving ^{90}Y-labeled anti-Tac showed a

prolongation of graft survival from a value of 6–8 days in untreated animals to a mean value of 40 days in animals receiving a dose of radioactivity that had acceptable toxicity (*102*). In light of these encouraging results, human recipients of cadaver donor renal allografts are receiving different anti-IL-2 receptor monoclonal antibodies as adjunctive immunotherapy (*108,109*). Antibody treatment has been well tolerated, and 50 of 53 recipients treated have retained a functioning allograft.

In summary, our present understanding of the IL-2–IL-2 receptor system opens the possibility for more specific immune intervention strategies. The IL-2 receptor may prove to be an extraordinarily versatile therapeutic target. The clinical applications of anti-IL-2 receptor-directed therapy represent a new perspective for the treatment of certain neoplastic diseases and autoimmune disorders and for the prevention of allograft rejection.

References

(*1*) KOHLER G, MILSTEIN C: Continuous cultures of fused cells secreting antibody of pre-defined specificity. Nature 256:495–497, 1975

(*2*) LEVY R, MILLER RA: Tumor therapy with monoclonal antibodies. Fed Proc 42:2650–2656, 1983

(*3*) CATANE R, LONGO DL: Monoclonal antibodies for cancer therapy. Isr J Med Sci 24:471–476, 1988.

(*4*) HOUGHTON AN, MINTZER D, CORDON-CARDO C, ET AL: Mouse monoclonal IgG3 detecting GD3 ganglioside: A phase I trial in patients with malignant melanoma. Proc Natl Acad Sci USA 82:1242–1246, 1985

(*5*) MASUI H, KAWAMOTO T, SATO JD, ET AL: Growth inhibition of human tumor cells in athymic mice by anti-epidermal growth factor receptor monoclonal antibody. Cancer Res 44:1002–1007, 1984

(*6*) LIOTTA LA, RAO CN, WEWER UM: Biochemical interactions of tumor cells with the basement membrane. Annu Rev Biochem 55:1037–1057, 1986

(*7*) MORGAN DA, RUSCETTI FW, GALLO RC: Selective in vitro growth of T lymphocytes from normal human bone marrows. Science 193:1007–1008, 1976

(*8*) SMITH KA: T-cell growth factor. Immunol Rev 51:337–357, 1980

(*9*) ROBB RJ, MUNCK A, SMITH KA: T-cell growth factor receptors: Quantification, specificity, and biological relevance. J Exp Med 154:1455–1474, 1981

(*10*) WALDMANN TA: The structure, function, and expression of interleukin-2 receptors on normal and malignant T cells. Science 232:727–732, 1986

(*11*) WALDMANN TA: The multisubunit IL-2 receptor. Annu Rev Biochem. In press

(*12*) UCHIYAMA T, BRODER S, WALDMANN TA. A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. J Immunol 126:1393–1397, 1981

(*13*) LEONARD WJ, DEPPER JM, UCHIYAMA T, ET AL: A monoclonal antibody that appears to recognize the receptor for human T-cell growth factor: Partial characterization of the receptor. Nature 300:267–269, 1982

(*14*) LEONARD WJ, DEPPER JM, ROBB RJ, ET AL: Characterization of the human receptor for T cell growth factor. Proc Natl Acad Sci USA 80:6957–6961, 1983

(*15*) LEONARD WJ, DEPPER JM, WALDMANN TA, ET AL: A monoclonal antibody to the human receptor for T-cell growth factor. *In* Receptors and Recognition (Greaves M, ed), vol 17. London: Chapman & Hall, 1984, pp 45–66

(*16*) SHACKELFORD DA, TROWBRIDGE IS: Induction of expression and phosphorylation of the human interleukin-2-receptor by a phorbol ester. J Biol Chem 259:11706–11712, 1984

(*17*) DEPPER JM, LEONARD WJ, KRÖNKE M, ET AL: Regulation of interleukin 2 receptor expression: Effects of phorbol diester, phospholipase C, and reexposure to lectin or antigen. J Immunol 133:3054–3061, 1984

(*18*) ROBB RJ, GREENE WC, RUSK C: Low and high affinity cellular receptors for interleukin 2. Implications in the level of Tac antigen. J Exp Med 160:1126–1146, 1984

(*19*) HATAKEYAMA M, MINAMOTO S, UCHIYAMA T, ET AL: Reconstitution of functional receptor for human interleukin 2 in mouse cells. Nature 318:467–470, 1985

(*20*) SABE H, KONDO S, SHIMUZEV A, ET AL: Properties of human interleukin-2 receptors expressed on non-lymphoid cells by cDNA transfection. Mol Biol Med 2:379–396, 1984

(*21*) GREENE WC, ROBB RJ, SVETLIK PB, ET AL: Stable expression of cDNA encoding the human interleukin 2 receptor in eukaryotic cells. J Exp Med 162:363–368, 1985

(*22*) ROBB RJ: Conversion of low affinity interleukin 2 receptor to a high affinity state following fusion of cell membranes. Proc Natl Acad Sci USA 83:3992–3996, 1986

(*23*) KONDO S, SHIMIZU A, SAITO Y, ET AL: Molecular basis for two different affinity states of the interleukin 2 receptor: Affinity conversion model Proc Natl Acad Sci USA 83:9026–9029, 1986

(*24*) REEM G, YEH N-H: Interleukin-2 regulates expression of its receptor and synthesis of gamma interferon by human T lymphocytes. Science 255:429–430, 1984

(*25*) WALDMANN TA, GOLDMAN CK, ROBB RJ, ET AL: Expression of interleukin-2 receptors on activated human B-cells. J Exp Med 160:1450–1466, 1984

(*26*) ORTALDO JR, MASON AT, GERARD JP, ET AL: Effects of natural and recombinant IL 2 on regulation of IFN-γ production and natural killer activity: Lack of involvement of the Tac antigen for these immunoregulatory effects. J Immunol 133:779–783, 1984

(*27*) GRIMM EA, ROSENBERG SA: The human lymphokine-activated killer cell phenomenon. *In* Lymphokines (Pick E, ed), vol 9. New York: Academic Press, 1984, pp 279–311

(*28*) WALDMANN TA, KOZAK RW, TSUDO M, ET AL. The role and molecular biology of the interleukin-2/interleukin-2 receptor system. *In* Progress in Immunology. VI (Cinidar B, Miller RG, eds). Orlando: Academic Press, 1986, pp 553–562

(*29*) TSUDO M, KOZAK RW, GOLDMAN CK, ET AL: Demonstration of a new (non-Tac) peptide that binds interleukin-2: A potential participant in a multichain interleukin-2 receptor complex. Proc Natl Acad Sci USA 83:9694–9698, 1986

(*30*) TSUDO M, KOZAK RW, GOLDMAN CK, ET AL: Contribution of a p75 interleukin-2 binding peptide to a high-affinity interleukin-2 receptor complex. Proc Natl Acad Sci USA 84:4215–4218, 1987

(*31*) WALDMANN TA: The multichain interleukin-2 receptor: From the gene to the bedside. Harvey Lect 82:1–17, 1988

(*32*) SHARON M, KLAUSNER RD, CULLEN BR, ET AL: Novel interleukin-2-receptor subunit detected by cross-linking under high-affinity conditions. Science 234:859–863, 1986

(*33*) DUKOVICH M, WANG Y, THUY LI, ET AL: A second human interleukin-2 binding protein that may be a component of high-affinity interleukin-2 receptors. Nature 327:518–521, 1987

(*34*) ROBB RJ, RUSK CM, YODOI J, ET AL: Interleukin-2 binding molecule distinct from the Tac protein: Analysis of the role in formation of high-affinity receptors. Proc Natl Acad Sci USA 84:2002–2011, 1987

(*35*) LOWENTHAL JL, GREENE WC: Contrasting interleukin-2 binding properties of the α (p55) and β (p70) protein subunits of the human high-affinity interleukin-2 receptor. J Exp Med 166:1156–1161, 1987

(*36*) WANG H-M, SMITH KA: The interleukin-2 receptor: Functional consequences of its biomolecular structure. J Exp Med 166:1055–1069, 1988

(*37*) SMITH KA: The interleukin-2 receptor. Adv Immunol 42:165–197, 1988

(*38*) MILLS GB, GIRARD P, GRINSTEIN S, ET AL. Interleukin-2 induces proliferation of T lymphocyte mutants lacking protein kinase C. Cell 55:91–100, 1988

(*39*) GAULTON G, EARDLEY D: Interleukin-2 dependent phosphorylation of interleukin-2 receptors and other T-cell membrane proteins. J Immunol 136:2470–2477, 1986

(*40*) BENEDICT SH, MILLS GB, GELFAND EW: Interleukin-1 activates a receptor-associated protein kinase. J Immunol 139:1694–1697, 1987

(*41*) SARAGOVI H, MALEK TR: γ chain of the IL-2 receptor: Identification of a putative novel peptide disulfide linked to the p75 β subunit. FASEB J 3: abstr 671, 1989

(*42*) SZÖLLÖSI J, DAMJANOVICH S, GOLDMAN CK, ET AL. Flow cytometric resonance energy transfer measurements support the association of a 95-kDa peptide termed T27 with the 55-kDa Tac peptide. Proc Natl Acad Sci USA 84:7246–7251, 1987

(*43*) EDIDIN M, ASZALOS A, DAMJANOVICH S, ET AL: Lateral diffusion measurements give evidence for association of the Tac peptide of

the IL-2 receptor with the T27 peptide in the plasma membrane of HUT 102 B2 T cells. J Immunol 141:1206–1210, 1988

(44) Herrmann F, Diamantstein T: The mouse high affinity IL-2-receptor complex. I. Evidence for a third molecule, the putative γ-chain associated with the α- and/or β-chain of the receptor. Immunobiology 175:145–158, 1987

(45) Saragovi H, Malek TR: The murine interleukin-2-receptor. Irreversible cross-linking of radiolabeled interleukin-2 to high affinity interleukin-2-receptors reveals a noncovalently associated subunit. J Immunol 139:1918–1926, 1987

(46) Sharon M, Gnarra JR, Leonard WJ: Identification of a distinct 100 kDa protein that interacts with the murine IL-2 receptor. FASEB J 3:559, 1989

(47) Hermann T, Diamantstein T: The human intermediate-affinity interleukin-2 receptor consists of two distinct, partially homologous glycoproteins. Eur J Immunol 18:1051–1057, 1988

(48) Sharon M, Siegel JP, Tosato G, et al: The human interleukin 2 receptor β chain (p70). Direct identification, partial purification, and patterns of expression on peripheral blood mononuclear cells. J Exp Med 167:1265–1270, 1988

(49) Nakamura Y, Inamoto T, Sugie K, et al: Mitogenicity and down-regulation of high-affinity interleukin-2 receptor by YTA-1 and YTA-2, monoclonal antibodies that recognize 75-kDa molecules on human large granular lymphocytes. Proc Natl Acad Sci USA 86:1318–1322, 1989

(50) Brandhuber BJ, Boone T, Kenny WC, et al: Three-dimensional structure of interleukin-2. Science 238:1707–1709, 1987

(51) Ju G, Collins L, Kimberlee LK, et al: Structure–function analysis of human interleukin-2: Identification of amino acid residues required for biological activity. J Biol Chem 262:5723–5731, 1987

(52) Collins L, Tsien WH, Seals C, et al: Identification of specific residues of human interleukin 2 that affect binding to the 70-kDa subunit (p70) of the interleukin-2 receptor. Proc Natl Acad Sci USA 85:7709–7713, 1988

(53) Leonard WJ, Depper JM, Crabtree GR, et al: Molecular cloning and expression of cDNAs for the human interleukin-2 receptor. Nature 311:626–631, 1984

(54) Cosman D, Cerretti DP, Larsen L, et al: Cloning, sequence and expression of human interleukin-2 receptor. Nature 312:768–771, 1984

(55) Nikaido T, Shimizu N, Ishida H, et al: Molecular cloning of cDNA encoding human interleukin-2-receptor. Nature 311:631–635, 1984

(56) Korsmeyer SJ, Greene WC, Cossman J, et al: Rearrangement and expression of immunoglobulin genes and expression of Tac antigen in hairy cell leukemia. Proc Natl Acad Sci USA 80:4522–4526, 1983

(57) Herrmann F, Cannistra SA, Levine H, et al: Expression of interleukin-2 receptors and binding of interleukin-2 by gamma interferon induced human leukemic and normal monocytic cells. J Exp Med 162:1111–1116, 1985

(58) Holter W, Goldman CK, Casabo L, et al: The expression of functional IL-2-receptors by lipopolysaccharide and interferon-2 stimulated human monocytes. J Immunol 138:2917–2922, 1987

(59) Rubin LA, Kurman CC, Biddison WE, et al: A monoclonal antibody, 7G7/B6, binds to an epitope on the human interleukin-2 (IL-2) receptor that is distinct from that recognized by IL-2 or anti-Tac. Hybridoma 4:91–102, 1985

(60) Tsudo M, Kitamura F, Migasaka M: Characterization of the interleukin-2 receptor β chain using three distinct monoclonal antibodies. Proc Natl Acad Sci USA 86:1982–1986, 1989

(61) Tsudo M, Goldman CK, Bongiovanni KF, et al: The p75 peptide is the receptor for interleukin-2 expressed on large granular lymphocytes and is responsible for the interleukin-2 activation of these cells. Proc Natl Acad Sci USA 84:5394–5398, 1987

(62) Uchiyama T, Nelson DL, Fleischer TA, et al: A monoclonal antibody (anti-Tac) reactive with activated and functionally mature human T cells. II. Expression of Tac antigen on activated cytotoxic killer T cells, suppressor cells and on one of two types of helper T cells. J Immunol 126:1398–1403, 1981

(63) Depper JM, Leonard WJ, Waldmann TA, et al: Blockade of the interleukin-2 receptor by anti-Tac antibody: Inhibition of human lymphocyte activation. J Immunol 131:690–696, 1983

(64) Tsudo MT, Uchiyama K, Takatsuki H, et al: Modulation of Tac antigen on activated human T-cells by anti-Tac monoclonal antibody. J Immunol 129:592–595, 1982

(65) Neckers LM, Cossman J: Transferrin receptor induction in mitogen-stimulated human T lymphocytes is required for DNA synthesis and cell division and is regulated by interleukin-2. Proc Natl Acad Sci USA 80:3494–3498, 1983

(66) Depper JM, Leonard WJ, Drogula C, et al: Interleukin-2 (IL-2) augments transcription of the IL-2 receptor gene. Proc Natl Acad Sci USA 82:4230–4234, 1985

(67) Takeshita T, Goto Y, Tada K, et al: Monoclonal antibody defining the second chain (p75) of interleukin-2 receptor. J Exp Med 169:1323–1332, 1989

(68) Waldmann TA, Greene WC, Sarin PS, et al: Functional and phenotypic comparison of human T cell leukemia/lymphoma virus positive adult T cell leukemia with human T cell leukemia/lymphoma virus negative Sézary leukemia, and their distinction using anti-Tac: Monoclonal antibody identifying the human receptor for T cell growth factor. J Clin Invest 73:1711–1718, 1984

(69) Uchiyama T, Hori T, Tsudo M, et al: Interleukin-2 receptor (Tac antigen) expressed on adult T-cell leukemia cells. J Clin Invest 76:446–453, 1985

(70) Schwarting R, Gerdes J, Stein H: Expression of interleukin-2 receptor on Hodgkin's and non-Hodgkin's lymphoma and macrophages. J Clin Pathol 38:1196–1197, 1985

(71) Ralfkiaer E, Wantzen GL, Stein H, et al: T-cell growth factor receptor (Tac antigen) expression in cutaneous lymphoid infiltrate. J Am Acad Dermatol 15:628–637, 1987

(72) Sheibani K, Winberg CD, Van de Velde S, et al: Distribution of lymphocyte with interleukin-2 receptors (Tac antigen) in reactive lymphoproliferative processes, Hodgkin's disease and non-Hodgkin's lymphoma. Am J Pathol 127:29–37, 1987

(73) Armitage RJ, Lai AP, Roberts PJ, et al: Certain myeloid cells possess receptors for IL-2. Br J Haematol 64:799–807, 1986

(74) Yamamoto S, Hattori T, Matsuoka M, et al: Induction of Tac antigen and proliferation of myeloid leukemic cells by ATL-derived factor: Comparison with other agents that promote differentiation of human myeloid or monocytic leukemia cells. Blood 67:1714–1720, 1986

(75) Nelson DL, Rubin LA, Kurman CC, et al: An analysis of the cellular requirements for the production of soluble interleukin-2 receptors in vitro. J Clin Immunol 6:114–120, 1986

(76) Waldmann TA: The multichain interleukin-2 receptor: A target for immunotherapy of patients with adult T-cell leukemia, autoimmune disorders, and individuals receiving allografts. J Autoimmun 1:641–655, 1988

(77) Keystone EL, Snow KM, Bombardier C, et al: Elevated soluble interleukin-2 receptor levels in the sera and sigmoid fluids of patients with rheumatoid arthritis. Arthritis Rheum 31:844–849, 1988

(78) Zoumbos NC, Gascon P, Djeu J, et al: Circulating activated suppressor T-lymphocytes in aplastic anemia. N Engl J Med 312:257–265, 1985

(79) Williams JM, Kelley VE, Kirkman RL, et al: T-cell activation antigens: Therapeutic implications. Immunol Invest 16:687–723, 1988

(80) Diamantstein T, Osawa H: The interleukin-2 receptor, its physiology, and a new approach to a selective immunosuppressive therapy by anti-interleukin-2 receptor monoclonal antibodies. Immunol Rev 92:5–27, 1986

(81) Lawrence EC, Brousseau KP, Kurman CC, et al: Soluble interleukin-2 receptor levels in serum as a marker of rejection in heart–lung transplantation. Chest 89(suppl):S526, 1986

(82) Takasuki K, Uchiyama T, Sagawa K, et al: Adult T cell leukemia in Japan. *In* Topics in Hematology (Seno S, Takaku F, Irino S, eds). Amsterdam: Excerpta Medica, 1977, pp 73–77

(83) Broder S, Bunn PA Jr, Jaffe ES, et al: NIH conference. T-cell lymphoproliferative syndrome associated with human T-cell leukemia/lymphoma virus. Ann Intern Med 100:543–557, 1984

(84) Poiesz BJ, Ruscetti FW, Gazdar AF, et al: Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. Proc Natl Acad Sci USA 77:7415–7419, 1980

(85) Seiki M, Hattori S, Hirayama Y, et al: Human adult T-cell leukemia virus: Complete nucleotide sequence of the provirus genome integrated in leukemia cell DNA. Proc Natl Acad Sci USA 80:3618–3622, 1983

(86) Sodroski JG, Rosen CA, Haseltine WA: *Trans*-acting transcriptional activation of the long terminal repeat of human T lymphotrophic viruses in infected cells. Science 225:381–385, 1984

(87) Paskavis H, Felber BK, Parlakis GN: *Cis*-acting sequences responsible for the transcriptional activation of human T-cell leukemia virus type I constitute a conditional enhancer. Proc Natl Acad Sci USA 83:6558–6562, 1986

(88) Shimotohno K, Miwa M, Slamon DJ, et al: Identification of new gene products coded from X regions of human T-cell leukemia viruses. Proc Natl Acad Sci USA 82:302–306, 1985

(89) SUZUKI N, MATSUNAMI N, KANAMORI H, ET AL: The human IL-2 receptor gene contains a positive regulatory element that functions in cultured cells and cell-free extracts. J Biol Chem 262:5079–5086, 1987

(90) RUBEN S, POTEAT H, TAN T-H, ET AL: Cellular transcription factors and regulation of IL-2 receptor gene expression by HTLV-I *tax* gene product. Science 241:89–92, 1988

(91) INOUE J, SEIKI M, TANIGUCHI T, ET AL: Induction of interleukin-2-receptor gene expression by p40^x encoded by human T-cell leukemia virus type I. EMBO J 5:2883–2888, 1986

(92) CROSS SL, FEINBERG MD, WOLF JB, ET AL: Regulation of the human interleukin-2 α chain promoter: Activation of a nonfunctional promoter by the transactivation gene of HTLV-I. Cell 49:47–56, 1987

(93) MARUYAMA M, SHIBUYA H, HARADA H, ET AL: Evidence for aberrant activation of the autocrine loop by HTLV-I encoded p40^x and T3-Ti complex triggering. Cell 48:343–350, 1987

(94) SIEKEWITZ M, FEINBERG MB, HOLBROOK N, ET AL: Activation of interleukin-2 and interleukin-2 receptor promoter expression by transactivator (tat) gene product of HTLV-I. Proc Natl Acad Sci USA 84:5389–5393, 1987

(95) FUJII M, SASSONE-CORSI P, VERMA IM: c-*fos* promoter *trans*-activation by the tax$_1$ protein of human T-cell leukemia virus type I. Proc Natl Acad Sci USA 85:8526–8530, 1988

(96) WALDMANN TA, LONGO DL, LEONARD WJ, ET AL: Interleukin 2 receptor (Tac antigen) expression in HTLV-I-associated adult T-cell leukemia. Cancer Res 45(suppl):4559s–4562s, 1985

(97) WALDMANN TA, GOLDMAN CK, BONGIOVANNI KF, ET AL: Therapy of patients with human T-cell lymphotrophic virus-I-induced adult T-cell leukemia with anti-Tac, a monoclonal antibody to the receptor for interleukin-2. Blood 72:1805–1816, 1988

(98) FITZGERALD DJP, WALDMANN TA, WILLINGHAM MC, ET AL: *Pseudomonas* exotoxin-anti-Tac: Cell-specific immunotoxin active against cells expressing the human T-cell growth factor receptor. J Clin Invest 74:966–971, 1984

(99) HWANG J, FITZGERALD DJ, ADYA S, ET AL: Functional domains of *Pseudomonas* exotoxin identified by deletion analysis of the gene expressed in *E coli*. Cell 48:129–136, 1987

(100) LORBERBOUM-GALSKI H, KOZAK RW, WALDMANN T, ET AL: IL-2-PE40 is cytotoxic to cells displaying either the P55 or P75 subunit of the IL-2-receptor. J Biol Chem 263:18650–18656, 1988

(101) KOZAK RW, ATCHER RW, GANSOW OA, ET AL: Bismuth-212 labeled anti-Tac monoclonal antibody: Alpha-particle emitting radionuclides as modalities for radioimmunotherapy. Proc Natl Acad Sci USA 83:474–478, 1986

(102) COOPER MM, ROBBINS RC, GANSOW OA, ET AL: Use of anti-Tac antibody in primate cardiac xenograft transplantation. Surg Forum 39:353–355, 1988

(103) KELLEY VE, GAULTON GN, HATTORI M, ET AL: Anti-interleukin-2-receptor antibody suppresses murine diabetic insulitis and lupus nephritis. J Immunol 140:59–61, 1988

(104) VOLK HD, BROCKE S, OSAWA H, ET AL: Effects of in vivo administration of a monoclonal antibody specific for the interleukin-2 receptor on the acute graft-versus-host reaction in mice. Clin Exp Immunol 66:126–131, 1986

(105) KIRKMAN RL, BARRETT LV, GAULTON GN, ET AL: Administration of an anti-interleukin-2-receptor monoclonal antibody prolongs cardiac allograft survival in mice. J Exp Med 162:358–362, 1985

(106) SHAPIRO ME, KIRKMAN RL, REED MH, ET AL: Monoclonal anti-IL-2-receptor antibody in primate renal transplantation. Transplant Proc 19:594–598, 1987

(107) BACHA P, WILLIAMS DP, WATERS C, ET AL: Interleukin 2 receptor-targeted cytotoxicity: Interleukin 2 receptor-mediated action of a diphtheria toxin-related interleukin 2 fusion protein. J Exp Med 167:612–622, 1988

(108) SOULILLOW JP, PEYRONNEL P, LEMAULT B, ET AL: Prevention of rejection of kidney transplants by monoclonal antibody directed against interleukin-2. Lancet 1:1339–1342, 1987

(109) SHAPIRO ME, KIRKMAN RL, CARPENTER CB, ET AL: Initial trials of the anti-human IL-2-receptor monoclonal antibody anti-Tac in clinical renal transplantation. Transplantation. In press

(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number: **0 162 699**
A2

(12) # EUROPEAN PATENT APPLICATION

(21) Application number: 85303575.6

(22) Date of filing: 21.05.85

(51) Int. Cl.⁴: C 12 N 15/00
C 12 P 21/02, C 07 K 13/00

(30) Priority: 21.05.84 US 612559
02.07.84 US 626666
02.07.84 US 626667
16.10.84 US 661346
13.11.84 US 670883

(43) Date of publication of application:
27.11.85 Bulletin 85/48

(84) Designated Contracting States:
AT BE CH DE FR GB IT LI LU NL SE

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(54) **Interleukin-2 receptor and DNA encoded therefor and their preparation.**

(57) Interleukin-2 receptor derived from normal and malignant cells has been purified by use of various techniques including affinity chromatography in conjunction with a monoclonal antibody directed to the receptor. The purification process also includes reversed phased high performance liquid chromatography. By these techniques, interleukin-2 receptor has been purified to homogeneity. The high purification of the interleukin-2 receptor has made possible the sequencing of the amino acid residues at the N-terminal of this protein molecule. Double-stranded cDNA is prepared from polyadenylated RNA extracted from cell lines or other sources known to produce IL-2 receptor. The cDNA is inserted within a plasmid vector and then the recombinant plasmid employed to transform an appropriate host. Transformed hosts are identified and grouped into pools. Plasmid DNA prepared from these pools is hybridized with a labeled synthetic oligonucleotide probe corresponding to a portion of the amino acid sequence of the purified IL-2 receptor. The cDNA clone isolated with the probe is characterized by restriction enzyme mapping and sequenced by chain-termination method. The particular DNA clone that actually contains the gene coding for the functional IL-2 receptor is identified by expressing the clones in COS-7 monkey kidney cells and assaying for the expressed IL-2 receptor by its ability to bind IL-2 or a monoclonal antibody directed against the IL-2 receptor.

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0162699

INTERLEUKIN-2 RECEPTOR AND DNA ENCODED THEREFOR AND THEIR PREPARATION

The present invention relates to interleukin-2 receptor (hereinafter "IL-2 receptor"), and more particularly to: purified interleukin-2 receptor derived from normal and malignant cells; a process for producing same; the cloning of IL-2 receptor gene by use of a synthetic oligonucleotide probe derived from the amino acid sequence of the purified IL-2 receptor to screen a complementary deoxyribonucleic acid ("cDNA") library synthesized from IL-2 receptor messenger ribonucleic acid ("mRNA"); and, the characterization of the screened IL-2 receptor gene.

A large number of normal immune responses require the participation of T-cells. The proliferation of T-cells to sufficient numbers to assume an effective role in immune responses is controlled by the presence of interleukin-2 (hereinafter "IL-2"), Gillis and Smith, 28 Nature 154 (1977). Although the mechanism by which IL-2 controls the growth of T-cells is not fully understood, it is known that IL-2 acts on T-cells via a specific, high-affinity, plasma membrane receptor, i.e., IL-2 receptor. Also, in order to continue to divide, IL-2 dependent T-cells must express the IL-2 receptor and the IL-2 must bind to a portion of the IL-2 receptor, Robb et al., 154 J. Exp. Med. 1455 (1981). A more complete knowledge of the biochemistry of the IL-2 receptor would foster a better understanding of the interaction between IL-2 and T-cells. To date, this has been hampered, at least in part, by the unavailability of sufficient amounts of IL-2 receptor in purified form.

Leonard et al., 300 Nature (London) 267 (November 1982), reported employing a murine monoclonal antibody, designated as anti-Tac, to significantly block the binding of radiolabelled IL-2 to the human lymphoma T-cell line, HUT-102. This antibody resulted from the immunization of mice with long term cultures of human T-cells. The anti-Tac antibody was reported as binding both

to a glyco-protein having a molecular weight of about 47,000-53,000 daltons and also to proteins having molecular weights of about 113,000 and 180,000 daltons. Leonard et al. hypothesized, but did not establish, that the cell surface determinant (i.e., the 47,000-53,000 molecular weight protein) to which the anti-Tac antibody bounded to was the IL-2 receptor.

Robb and Green, 158 J. Exp. Med. 1332 (1983), reported employing the anti-Tac antibody in conjunction with mitogen-activated normal lymphocytes to immunoprecipitate a protein having a molecular weight of about 52,000-57,000 daltons. They found that this same protein also bound to IL-2. These researchers opined that this reactive molecule contained the binding site for IL-2 for normal lymphocytes.

Leonard et al., Proc. Natl. Acad. Sci. (USA) 6957 (1983) observed that receptors recognized by anti-Tac antibody on HUT-102 cells and on phytohemaggelutinin - activated normal T-cells appear to be larger on reducing gels than on nonreducing gels, thus suggesting the presence of intrachain disulfide bonds. Also, the HUT-102 cell receptor was reported to exhibit an isoelectric point of from 5.5 to 6.0. From post-translational studies, Leonard et al. suggested that the HUT-102 receptor is composed of a peptide backbone of 33,000 daltons that is initially glycosylated by an N-linked mechanism to achieve a 35,000-37,000 daltons doublet and then glycosylated by an O-linked mechanism to increase the weight of the molecule by about 13,000-15,000 da tons. Although the researchers stated that their studies "suggested" that the HUT-102 cell receptor recognized by the anti-Tac antibody is the human receptor for IL-2, they admitted that actual proof would require purifying the receptor, which prior to the making of the present invention had not been accomplished.

Recombinant DNA techniques have been developed for economically producing a desired protein once the gene coding for protein has been isolated and identified. A discussion of such recombinant DNA techniques for protein production is set forth in the editorial and supporting papers in Vol. 196 of Science (April, 1977). However, to take advantage of the recombinant DNA techniques discussed in these references, the gene coding for the IL-2 receptor must first be isolated.

The present invention relates to the production of IL-2 receptor derived from malignant and normal T-cells, to the purification of the IL-2 receptor to homogeneity and to the determination of the amino acid sequence of the amino terminal portion of the IL-2 receptor molecule. The IL-2 receptor of the present invention is purified by a combination of affinity chromatography

and reversed phased high performance liquid chromatography. The affinity chromatography procedure employs a highly specific monoclonal antibody that recognizes an epitope on the receptor molecule.

Once purified to homogeneity, the amino acid sequence of the amino terminal portion of the receptor molecule can be ascertained by use of a protein sequencer. This information is used to construct a hybridization probe to isolate the IL-2 receptor from a cDNA library constructed from mRNA receptors isolated from cells known to express IL-2. To this end, total RNA is extracted from cell lines or other sources known to produce relatively high levels of IL-2 receptor molecules. Polyadenylated mRNA is isolated from the total RNA extract. A cDNA library is constructed by reverse transcription of the polyadenylated mRNA with reverse transcriptase. The DNA is rendered double-stranded with DNA polymerase I and inserted into a cloning vector, and the recombinant vector is used to transform a host.

Transformed hosts are identified and grouped into pools. Plasmid DNA prepared from these pools is hybridized with a labeled synthetic oligonucleotide probe corresponding to a portion of the amino acid sequence of the IL-2 receptor. The pool(s) of clones that give a positive signal to the probe are identified, replated as single colonies, and hybridized with the synthetic oligonucleotide probe to identify the particular host colony containing the IL-2 receptor gene. Plasmid DNA is prepared from this host colony and characterized by restriction enzyme mapping. The IL-2 receptor gene is sequenced to establish its entire nucleotide and amino acid composition. In addition, the IL-2 receptor gene is cloned in a mammalian cell system to express mature IL-2 receptor and then a binding assay is conducted to confirm that the expressed protein product is the IL-2 receptor.

The details of typical embodiments of the present invention will be described in connection with the accompanying drawings, in which:

FIGURE 1 illustrates partial restriction maps of the IL-2 Rec N4 ("N4") and IL-2 Rec N1 ("N1") clones in side-by-side comparative relationship to each other;

FIGURE 2 illustrates the nucleotide sequence and the corresponding amino acid sequence of the IL-2 receptor gene as contained in the N4 nucleotide fragment, with the nucleotides being numbered from the position of the initiator methionine codon and the amino acids being numbered from the mature NH_2-terminus of the protein, i.e., the Glu residue, as marked with a star;

FIGURE 3A illustrates the strategy employed to clone the coding regions of the N4 and N1 fragments in plasmid vectors used to transfect mammalian cells to determine whether one or both cDNA clones would encode a functional IL-2 receptor; and

FIGURES 3B and C illustrate the ability of the transfected mammalian cells to bind to IL-2 and to a monoclonal anti-IL-2 receptor antibody.

Preparation of IL-2 Receptor Extracts From Malignant and Normal Cells

Malignant cells are cultured in vitro in a suitable culture medium supplemented with serum and various additives. After an optimum culture period, the cells are harvested and IL-2 receptor containing extracts formed from the cells. The malignant cell lines which may be employed as a source of IL-2 receptors include T-lymphoma or T-leukemia cell lines. These cell lines are produced by either a spontaneous occurrence, via viral transformation or via transformation by chemical carcinogen or irradiation. The present invention has been carried out in conjunction with a naturally occurring lymphoma cell line, designated as HUT-102. The cell line is available from a wide variety of sources and has been used extensively by researchers. See, for instance, Leonard et al., 80 Proc. Natl. Acad. Sci (USA), 6959 (1983) and Leonard et al., 300 Nature (London), 267 (November 1982).

The present invention also includes producing IL-2 receptor molecules from normal cells. For instance, human peripheral blood mononuclear cells are separated from human blood by Ficoll-Hypaque centrifugation, such as described by Boyum, 18 Scand. J. Clin. Lab. Invest. Suppl. 77 (1966). Adherent cells are removed by plastic adherence and then nonadherent cells are cultured in vitro in serum containing medium in the presence of an activating agent, such as a T-cell mitogen. After a suitable period of time, the cells are harvested by centrifugation. Examples of T-cell mitogens that may be used as activating agents, include phytohemagglutinin ("PHA"), concanavalin A ("Con A") or pokeweed mitogen ("PKM").

The numbers of IL-2 receptors expressed by stimulation of the peripheral blood leukocytes with a plant mitogen varies with time. Optimum levels of IL-2 receptor expression are reached at approximately 72 hours after mitogen stimulation.

The culture medium used to expand the IL-2 receptor bearing malignant and normal cells may consist of commercially available medium, such

as Roswell Park Memorial Institute ("RPMI") medium. Dulbecco's Modified Eagle Medium ("DMEM") and Click's Medium. Additives, which may be individually or in combination added to the culture medium, include serum, such as fetal calf serum ("FCS") or normal human serum. Additional additives include glutamine and various antibiotics, such as penicillin and streptomycin.

The process of culturing the malignant and normal cells to induce receptor formation may be carried out in various environmental conditions. Preferably, however, the cultures are maintained in the temperature range of approximately 35-38°C in a humidified atmosphere of approximately 5-10% CO_2 in air. Also, the pH of the culture should be kept in slightly alkaline condition, in the range of approximately pH 7.0-7.4.

IL-2 receptor containing extracts are prepared from the cultivated normal and malignant cells by harvesting the cells by centrifugation. The cells are then washed with a buffered saline solution and resuspended in the buffered saline solution together with a detergent and phenylmethylsulfonylfluoride ("PMSF"). After a period of time the detergent extract is centrifuged to remove nuclei and insoluble debris and then is stored frozen until used.

Preparation of Monoclonal Antibody Against IL-2 Receptor

The present invention also concerns the production of a monoclonal antibody having a high affinity to an epitope on the IL-2 receptor molecule. The antibody is used as a bound ligand in the affinity chromatography procedures during purification of the IL-2 receptor. The antibody is also employed in a radioimmune precipitation assay and in soluble receptor assays to monitor the IL-2 receptor protein during purification steps, as more fully discussed below.

A preferred procedure for generating the monoclonal antibody against the IL-2 receptor is generally disclosed in U.S. Patent 4,411,993, incorporated herein by reference. In the procedure, BALB/c mice are injected with PHA activated human peripheral blood leukocytes ("PHA/PBL") several times at weekly intervals. Prior to the first injection, the PHA/PBL is emulsified in complete Freund's adjuvant and prior to the remainder of the injections the PHA/PBL is emulsified in incomplete Freund's adjuvant.

During the course of immunization, serum samples from the mice are tested by an enzyme linked immunoabsorbant assay ("ELISA"), as is well known in the art, for the presence of antibody reaction with the immunization cells. Once an antibody titer is detected, the animals are given an intravenous injection of PHA/PBL suspended in saline. Several days later the animals are sacrificed and their spleens harvested. Single cell suspensions from the splenocytes are cultured in tissue culture medium supplemented with various

additives to expand the number of antibody producing cells. The antibody producing cells are isolated from the culture and purified by standard techniques for subsequent fusion with myeloma cells to produce hybrid cells that express anti-IL-2 receptor antibody. The fusion process is detailed in U.S. Patent 4,411,933 and in Nowinski et al., 93 Virology 111 (1979), incorporated herein by reference.

After fusion, the hybrid cells are resuspended in a tissue culture medium supplemented with various additives and selected suppressing agents to preclude the growth of unfused myeloma cells, double myeloma cells, unfused spleen cells and double spleen cell hybrids, thereby allowing the anti-IL-2 receptor antibody producing cells to grow. Such growth inhibitors or suppressants may include hypoxanthine, aminopterin and thymidine (hereinafter collectively referred to as "HAT").

After several days of culture, the hybridoma cells, which are generated, are screened by ELISA assay for anti-IL-2 receptor antibody responses. These hybrid cells are tested for production of antibody capable of inhibiting both mitogen and antigen induced proliferation of human peripheral blood leukocytes. The hybrid cells which give positive ELISA results are gradually weaned to HAT-free medium and then cultured in vitro in large volumes for bulk production of antibody. Alternatively, the cells may be expanded in vivo by injecting the hybridoma cells in the peritoneal cavities of mice and thereafter collecting the intraperitoneal ascites which contain high concentrations of the antibody. The antibodies contained in the ascites fluid can be isolated and concentrated by established techniques, such as by ammonium sulfate precipitation followed by gel column chromatography. If required, the antibody can be further purified by ion exchange chromatography and/or affinity chromatography. By the above process, a particular hybridoma, designated as 2A3, was found to produce antibody that significantly inhibited both mitogen and antigen induced proliferation of human peripheral blood leukocytes.

The present invention also includes identifying potent cell line sources of anti-IL-2 receptor antibody by cloning cell lines known to produce this antibody, for instance, the 2A3 cell line. The cloning is accomplished by the limiting dilution procedure, as is well known in the art and as is detailed in U.S. Patent No. 4,411,993. By this procedure, one particular subclone, designated as 2A3-A1H was found to produce antibody that substantially entirely inhibited both mitogen and antigen induced proliferation of human peripheral blood leukocytes. The 2A3-A1H antibody has been characterized as of the $_1$K isotype with an unusually high affinity to the human IL-2 receptor.

A control antibody preferably is employed to confirm the processes of the present invention utilizing anti-IL-2 receptor antibody and as a reagent in the purification of the receptor. The control antibody should be of the same isotype as the anti-IL-2 receptor antibody. Applicants have identified the antibody secreted by the mouse myeloma cell line MOPC-21 as a suitable control antibody for the 2A3-A1H antibody. The MOPC-21 cell line is widely available from numerous private and commercial sources.

Soluble IL-2 Receptor Assays

Assays employing the 2A3-A1H monoclonal antibody are used in conjunction with the present invention to monitor the quantitative amount of IL-2 receptor present in the initial cell lysates and during purification procedures. These assays hinge on the discovery by applicants that the 2A3-A1H antibody has an extremely high affinity for the IL-2 receptor, the affinity constant being in excess of 5×10^9 M^{-1} and that the 2A3-A1H antibody can be radioiodinated to high specific activity and still retain its capacity to bind to the IL-2 receptor.

One such perferred assay involves ascertaining the extent to which samples of cell lysate or column chromatography fractions containing IL-2 receptors are capable of inhibiting the binding of radiolabelled IL-2 antibody to glutaraldehyde fixed, intact receptor bearing cells. This assay relies on the observation by applicants that IL-2 receptor is stable to glutaraldehyde fixation, i.e., the receptor cannot be extracted from such cells with nonionic detergents, such as Triton X-100, and the presence of detergent does not affect the binding of radiolabelled 2A3-A1H antibody to the fixed cells. Preincubation of a subsaturating dose of iodinated 2A3-A1H antibody with detergent solutions containing the IL-2 receptor inhibits the subsequent binding of the 2A3-A1H antibody to the glutaraldehyde fixed cells. This assay will hereinafter be referred to as the "soluble inhibition assay."

For use in the soluble inhibition assay, the 2A3-A1H antibody is radiolabeled with iodine 125 ("^{125}I") by a chloramine-T method, as is well known in the art and as described by Segal and Hurwitz, 118 J. Immunol. 1338 (1977). The standard labeling conditions employed are: 50 micrograms ("ug") 2A3-A1H IgG; 4 nanomoles ("nM") of chloramine-T (Sigma Chemical Company, St. Louis, Missouri); and, 2.5 microcurins ("mCi") of ^{125}I sodium iodide (New England Nuclear, Boston, MA), in a final volume of 60 microliters ("ul"). This protocol has resulted in preparations of ^{125}I-2A3-A1H, which routinely have specific activities in the range of 2 to 5×10^{15} counts per minute/millimole ("cpm/mMol") (1.3 - 3.3 x 10^7 cpm/ug). Also, 2A3-A1H antibodies labeled in this

way were found to be more than 95 percent bindable to IL-2 receptor bearing cells and had apparent affinity constants in excess of 5×10^9 M^{-1}.

In the soluble inhibition assay, 50 ul of ^{125}I-2A3-A1H [(2×10^{-10}M in RPMI - 1640 medium containing 2% bovine serum albumin ("BSA"), 20 mM HEPES buffer (pH 2.7) and 0.2% sodium azide ("NaN_3") (collectively "binding medium")] is mixed with 50 ul of cell lysate or column fraction diluted in phosphate buffered saline ("PBS") containing 1% (w/v) Triton X-100 detergent (Sigma Chemical Company, St. Louis, MO). This mixture is incubated for one hour at room temperature in round bottom 96 well plates (Linbro, Hamden, CT). At the end of the incubation period, 10^7 glutaraldehyde fixed, PHA activated human T-cells in 50 ul of binding medium are added to detect uncomplexed ^{125}I-2A3-A1H. Incubation is continued for one hour at room temperature. Duplicate 60 ul aliquots of the mixture are then transferred to precooled 400 ul polyethylene centrifuge tubes containing 200 ul of a phthalate oil mixture and the cell bound antibody is separated from unbound antibody by centrifugation. The details of the well-known phthalate oil separation method are set forth in Segal and Hurwitz, supra. The percent of specific inhibition caused by the lysate or column fraction is calculated by using 50 ul of PBS-2% Triton X-100 instead of a test sample for the positive control. Also, 15 ul of PBS-2% Triton X-100 containing 10^{-8} M unlabeled 2A3-A1H is used as a negative control.

The nitrocellulose dot assay ("dot assay") is used as a second soluble IL-2 receptor assay to quantify the amount of IL-2 receptor molecules present in a sample of cell lysate or column fraction. Briefly, in the dot assay, solutions are made of a $\log_2$ dilution series of potential IL-2 receptor containing samples and PBS containing 1% Triton X-100. Samples of 5 ul of these solutions are then applied to a piece of dry nitrocellulose (Schleicher and Schuell, Keene, N.H.). The nitrocellulose is then blocked by overnight incubation in 10 ml of 0.5 M TRIS, (pH 7.5), 0.15 M NaCl, 3% BSA (hereinafter TBS-3% BSA). After the blocking step, the nitrocellulose is incubated for one hour at room temperature in 10 ml of TBS-3% BSA containing 0.05 ug/ml ^{125}I - 2A3-A1H and 0.6 ug/ml unlabeled MOPC-21. The nitrocellulose is then washed three times in TRIS buffered saline and twice in TRIS buffered saline containing 1% (w/v) Nonidet P-40 detergent (Gallard Schlesinger Chemical Manufacturing Corp., Carle Place N.Y.), 1% (w/v) sodium deoxycholate, and 0.1% (w/v) sodium lauryl sulfate. Each of these washes lasts 30 minutes at room temperature. After the final wash, the nitrocellulose sheet is blotted dry, covered with a clear plastic sheet and then exposed at -70°C to Kodak X-omat AR^R film.

Radioimmune Precipitation Assay

The specificity of the IL-2 receptor antibody is ascertained with a radioimmune precipitation assay involving forming precipitations between samples of radiolabeled IL-2 receptor molecules and an antibody to the receptor and then employing polyacrylamide gel electrophoresis and either fluorography or autoradiography to visualize the receptor proteins that were precipitated. In this assay technique, the IL-2 receptor molecules are labeled either by surface iodination metabolically before extraction.

A surface podination of the IL-2 receptor on cell membranes after extraction is performed by the ^{125}I-IODO-GEN method (Pierce Cl. Co., Rockford, Illinois). The details of this radiolabeling technique are well known in the art and described by Urdal et al., 1 Cancer Metastasis Reviews 65 (1982); and, Markwell et al., 17 Biochemistry (Wash.) 4807 (1978). The use of ^{35}S methionine to label the receptor molecules metabolically also is well known in the art and is described by, for instance, Robb and Greene, supra.

After labeling with ^{125}I or ^{35}S methionine, the cells are washed with PBS and then extraeted with PBS containing 1% Triton X-100 and 2 mM PMSF. Affinity supports for the radioimmune precipitation assay are prepared by coupling purified antibodies (2A3-A1H and MOPC-21) to Affi-gel-10. Briefly, one volume of moist Affi-gel-10 is added to one volume of antibody (3-5 mg/ml) in borate buffered saline ("BBS") and then the mixture incubated overnight at 4°C. Thereafter, 100 ul of 1 M glycine ethylester is added per ml of gel to couple any of the unreacted groups on the Affi-gel-10. Applicants have found that routinely from 3 to 4 mg of antibody are coupled per ml of the gel under these conditions. Before use, each gel is washed extensively with PBS. Each gel is also washed with a buffer solution composed of PBS-1% Triton X-100 and 0.5 M TRIS, pH 7.5, containing 0.5 M NaCl, 1% (w/v) NP 40 detergent, 1% (w/v) sodium deoxycholate, and 0.1% sodium dodecyl sulfate ("SDS") (collectively "RIPA buffer").

The radioimmune precipitations are performed by mixing 50 ul of radiolabeled cell extract with 75 ul of PBS-1% percent Triton X-100 containing 20% (v/v) of affinity gel having antibody coupled thereto. The mixture is incubated over night at 4° C and then the gel washed four times with RIPA buffer and twice with 0.1 M TRIS, pH 8.0, containing 0.5 M NaCl, 5 mM, ethylene diamine tetra acetate ("EDTA"), and 0.5% NP-40 detergent. After the final wash, the resulting gel pellets are suspended in 40 ul of SDS polyacrylamide gel sample buffer (0.06 M TRIS, pH 6.8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol and boiled for three minutes to break apart the bonds

between the antibody and the IL-2 receptor molecules. A 30 ul sample of the supernate is then analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) (8-12% polyacrylamide gel for ^{125}I labeled receptor; 12% polyacrylamide gel for ^{35}S methionine labeled receptor) according to the stacking gel procedure of Laemmli, 227 Nature (London) 680 (1970).

In the lysate analysis the receptor proteins employing the ^{35}S methionine gels are visualized by fluorography. To this end, the ^{35}S methionine gels were impregnated with Enhance (New England Nuclear, Boston, MA) prior to drying and fluorography. The receptor proteins immunoprecipitated with the ^{125}I gels are visualized by autoradiography. To this end, the ^{125}I gels are stained with Coomassie blue prior to drying and autioradiography. Both the ^{35}S methionine and ^{125}I gels are exposed to Kodak X-omat AR^{R} film at -70°C for 24 to 72 hours.

Gel Electrophoresis of Chromatogrpahy Column Fractions

Fractions eluted from the affinity chromatography and reversed phase HPLC columns employed in the purification processes of the present invention are assayed by gel electrophoresis. 50 ul aliquots are removed from the eluate fractions. The aliquots are dried under vacuum after addition of 2 ul of 10% SDS (w/v) to each aliquot. The dried residue is dissolved in 40 ul of SDS polyacrylamide gel sample buffer and then boiled for 3 minutes. The solution is applied to an 8% polyacrylamide gel and electrophoresis is then carried out by the stacking gel procedure of Laemmli, supra. The resulting gel samples are silver stained by the method described by Oakley et al., 105 Anal. Biochem. 361 (1980).

Purification of IL-2 Receptor

Cell extracts from the malignant and normal cells produced by the above procedures are initially concentrated by affinity chromatography techniques employing the same affinity supports used in the radioimmune precipitation assay described above. The procedure employed involves applying cell extracts first to an MOPC-21 column and then to a second column prepared with a mixture of MOPC-21 antibody and 2A3-A1H antibody so that in the second column from 3 to 4 mg of total lgG is coupled to each ml of gel, but only 10 to 30% of the antibody is composed of 2A3-A1H. This technique is used to counteract the extremely high affinity between the 2A3-A1H antibody and the IL-2 receptor.

In the purification procedure, the cell extracts, as prepared above, are first applied to the MOPC-21 column that has been preequilabrated with a buffer containing a detergent, thereby to remove proteins in the cell extract

that might nonspecifically bind to mouse immunoglobulin. The flow through from the MOPC-21 column is then applied to the 2A3-A1H column. Elution from this column is carried out with a guanidine-HCL detergent solution. The recovered fractions are then dialyzed against decreasing concentrations of the eluting agent to optimize the recovery of biological activity.

Fractions are collected and assayed by gel electrophoresis and silver staining, as described above. Applicants have found that by use of the affinity chromatography procedure, IL-2 receptor from malignant cells which constitutively produce the receptor is purified approximately 1600 times from initial cell lysate. A somewhat lower purification level is typically attained for IL-2 receptor from activated normal cells.

The pooled active fractions from the above affinity chromatography procedure is employed as a starting material for the HPLC procedures. The HPLC technique used in the present invention preferably employs a reversed phase, tetra methyl bonded silica column having a pore size sufficiently large to be optimumly utilized with the proteineaceous IL-2 receptor, i.e., a pore size of at least 300 Å.

Suitable reversed phased HPLC columns for use in the practice of the present invention are articles of commerce. A preferred column for this purpose is the Vydac C-4 reversed phase column commercially available from Separations Group, Hesperia, CA. This column consists of tetra methyl silane groups covalently bonded by means of a siloxane (silicon-oxygen-silicon) bond to the surface of the 300 Å pore diameter silica gel which has been classified to a mean particle size of 5 microns. Alternative HPLC columns which may be employed in the present invention include those constructed with octylsilane (Vydac C-8) or octyldecylsilane (Vydac C-18) resins covalently bonded to silica gel.

The elution of the proteins from the HPLC column is carried out in a manner well known in the art. A suitable elution procedure for removing the bonded receptor molecule proteins from the tetra methyl column involves the use of a linear gradient of acetonitrile. A preferred gradient for this purpose is 0 to 95 percent (v/v) acetonitrile gradient in 0.1% (v/v) trifluoroacetic acid (TFA), pH 2.1.

The eluted protein can be conveniently monitored with detection systems that are well known in the art. The relative protein concentration in the fractions eluted from the HPLC columns can be determined by measuring absorbance of the eluded material in an automated ultraviolet light spectrophotometer, at 214 nanometers light wave length. The suitable

automated ultraviolet light absorbance detection apparatus is available from Waters Associates, Millford, MA. Final identification of the IL-2 receptor is dependent on the detection of the receptor by use of the soluble receptor assay and by use of gel electrophoresis as described above.

By use of the above-described soluble receptor assay techniques, applicants have found that the specific activity of the IL-2 receptor after HPLC purification is very high, i.e., approximately 21,000 fmole IL-2 receptor/ug protein for IL-2 receptor derived from malignant cells. This is approximately a 16,700 fold level of purification over the specific activity of the IL-2 receptor in the starting cell lysate. The specific activity of the IL-2 receptor from normal T-cells was about 1/3 of the specific activity from malignant cells. By polyacrylamide gel electrophoresis and silver staining, applicants ascertained that the molecular weight of the IL-2 receptor from normal cells is approximately 60,000 daltons, as opposed to 55,000 daltons for receptor molecules found constitutively on the malignant cells.

Amino Acid Sequencing

The ability to prepare homogeneous IL-2 receptor has permitted applicants to determine the amino acid sequence of the amino terminal portion of this molecule. This information may be employed to assist in the cloning of the IL-2 receptor gene and the production of large quantities of pure IL-2 receptor for clinical trials and ultimately for widespread medical use. Moreover, the availability of homogeneous IL-2 receptor will no doubt lead to a more complete understanding of the biology of IL-2. While the prior art has said to have partially "characterized" the IL-2 receptor, applicants are not aware of any instances in which this protein has been truly purified to homogeneity to the extent that the receptor can be analyzed for amino acid composition and sequence.

Samples of homogeneous IL-2 receptor, as prepared above, can be analyzed for amino acid composition and sequence, for instance with an automated sequencer, such as with an Applied Biosystems model 470A protein sequencer. Ideally, several sequencing runs are made to confirm the accuracy of the sequence. Through this technique, applicants have found that the first 15 residues of the amino terminal portion of the IL-2 receptor molecule are composed of the following sequence: Glu-Leu-Cys-Asp-Asp-Asp-Pro-Pro-Glu-Ile-Pro-His-Ala-Thr-Phe.

Sources of IL-2 Receptor Producing Cells

Preferably, a cDNA library, from which the gene coding for the IL-2 receptor will be sought, is constructed from cells known to produce high

levels of IL-2 receptor. As noted above, these sources may include malignant cell lines that have previously been identified as high level IL-2 receptor producers, such as the human lymphoma T-cell line designated as HUT-102, and human peripheral blood mononuclear cells.

Preparation of RNA from IL-2 Receptor Bearing Cells

Total RNA from the IL-2 receptor bearing cells is extracted by standard methods, such as disclosed by Chirgwin et al., 18 Biochemistry 5294 (1979) and Maniatis et al., Molecular Cloning, a Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1982).

As is well known, when extracting RNA from cells, it is important to minimize ribonuclease ("RNase") activity during the initial stages of extraction. One manner in which this is accomplished is to denature the cellular protein, including the RNase, at a rate that exceeds the rate of RNA hydrolysis by RNase. In the procedures of Chirgwin et al., supra, and Maniatis et al., supra at 196, this is carried out by use of guanidinium thiocyanate, together with a reducing agent, such as 2-mercaptoethanol (to break up the protein disulfide bonds). The RNA is isolated from the protein by standard techniques, such as phenol/chloroform extraction, ethanol precipitation or sedimentation through cesium chloride.

Although several techniques have been developed to separate the polyadenylated mRNA from the extracted protein, one preferred method is to chromatograph the polyadenylated mRNA on oligo (dT)-cellulose in the well known manner described by, for instance, Edmonds et al., 68 Proc. Natl. Acad. Sci. (USA) 1336 (1971); Aviv and Leder, 69 Proc. Natl. Acad. Sci. (USA) 1408 (1972); and Maniatis et al., supra at 197. The oligo (dT)-cellulose column is prepared with a loading buffer and then the mRNA applied to the column. Thereafter, the column is initially washed with a buffer solution to remove the unpolyadenylated mRNA and then the polyadenylated mRNA is eluted from the column with a buffered, low ionic strength, eluent. The integrity of the polyadenylated mRNA is verified by gel electrophoresis.

Preparation of cDNA from mRNA

A library of double-stranded cDNA corresponding to the mRNA is prepared by known techniques employing the enzyme reverse transcriptase. One such procedure which may be employed in conjunction with the present invention is detailed by Maniatis et al., supra at 230. Briefly, the polyadenylated mRNA is reverse transcribed by using oligo-dT that has been hybridized to the polyadenylated tail of the mRNA, as a primer for a first cDNA strand. This

results in a "hairpin" loop at the 3' end of the initial cDNA strand that serves as an integral primer for the second DNA strand. Next, the second cDNA strand is synthesized using the enzyme DNA polymerase I and the hairpin loop is cleaved by S1 nuclease to produce double-stranded cDNA molecules. The double-stranded cDNA is fractionated by any convenient means to remove the shorter strands thereby avoiding the needless cloning of small cDNA fractions.

It is to be understood that in accordance with the present invention, alternative well known procedures may be employed to prepare double-stranded cDNA from mRNA. One such alternative technique is disclosed by Land et al., 9 Nucl. Acids Res. 2251 (1981). In the Land et al. protocol, the hairpin loop is not used as a primer for the second cDNA strand. Rather, the 3' end of the first cDNA strand is tailed with dCMP residues using terminal deoxynucleotidyl transferase ("TdT"). This produces a 3' tail of poly-C residues. Then the synthesis of the second strand is primed by oligo-dG hybridized to the 3' tail. This technique is said to help avoid losing portions of the 5' tail of the second cDNA strand which might occur if the hairpin is cleaved with S1 nuclease, as in the Maniatis et al. protocol.

Cloning of cDNA

Next, the double-stranded cDNA is inserted within a cloning vector which is used to transform compatible prokaryotic or eukaryotic host cells for replication of the vector. Thereafter, the transformants are identified and plasmid DNA prepared therefrom.

To carry out the present invention, various cloning vectors may be utilized to clone the cDNA. Although the preference is for a plasmid, the vector may be a bacteriophage or a cosmid. If cloning occurs in mammalian cells, viruses also can be used as vectors.

If a plasmid is employed, it may be obtained from a natural source or artificially synthesized. The particular plasmid chosen should be compatible with the contemplated transformation host, whether a bacteria such as Escherichia coli ("E. coli"), yeast, or other unicellular microorganisms. The plasmid should have the proper origin of replication for the particular host cell to be employed. Also, the plasmid should have a phenotypic property that will enable the transformed host cells to be readily identified and separated from cells that do not undergo transformation. Such phenotypic characteristics can include genes providing resistance to growth inhibiting substances, such as an antibiotic. Plasmids are widely available that encode genes resistant to various antibiotics, such as tetracycline, streptomycin, sulfa drugs, penicillin, and ampicillin.

If E. coli is employed as the host cell, many possible cloning plasmids are commercially available which may be used in conjunction with the present invention. A preferred plasmid for performing the present invention is pBR322. This plasmid is widely commercially available and has been fully sequenced, as set forth in Sutcliffe, 43 Cold Spring Harbor Symp. Quant. Biol. 77 (1979). A significant advantage of this plasmid is that it has 11 known unique restriction sites at which the plasmid may be cleaved by a specific enzyme, including the Pst I site in the ampicillin resistant gene. This feature is particularly useful for cloning by the homopolymer tailing method.

If a bacteriophage is used instead of a plasmid, such phages should have substantially the same characteristics noted above for selection of plasmids. This includes the existence of a phenotypic marker and ligatable termini for attachment of foreign genes.

The double-stranded cDNA prepared from mRNA, having blunt ends, may be inserted within a plasmid cloning vector by various methods that are well-known in the art. One such technique involves attaching linkers to the ends of the cDNA strands. The linkers are composed of approximately 8 to 10 base pair oligonucleotides that are synthesized chemically and added to the double-stranded cDNA by using DNA ligase. The linkers are then cleaved with a restriction enzyme to generate cohesive termini for insertion within a plasmid cleaved with the same restriction enzyme.

An alternative procedure, and of preference in the present invention, is to insert the double-stranded cDNA into a plasmid vector by homopolymeric tailing. In this technique, complementary homopolymer tracks are added to the strands of the cDNA and to the plasmid DNA. The vector and double-stranded cDNA are then joined together by hydrogen bonding between complementary homopolymeric tails to form open, circular hybrid molecules capable of transforming host cells, such as E. coli.

In one procedure for homopolymeric tailing, approximately 50 to 150 dA nucleotide residues are added to the 3' ends of linearized plasmid DNA. A similar number of dT nucleotide residues are added to the 3' ends of the double-stranded cDNA and then the cDNA and plasmid joined together.

In an alternative tailing method, dG tails are added to the 3' ends of the cloning vector that has been cleaved with an appropriate restriction enzyme. For instance, if the pBR322 plasmid is employed, the restriction enzyme Pst I may be used to digest the plasmid at the ampicillin resistant gene. Complementary dC tails are added to the 3' ends of the double-stranded cDNA

prior to insertion of the cDNA segment in the plasmid with an appropriate annealing buffer.

The recombinant DNA plasmids, as prepared above, are used to transform host cells. Although the host may be any appropriate prokaryotic or eukaryotic cell, preferably, it is a well-defined bacteria, such as E. coli or a yeast strain. Such hosts are readily transformed and capable of rapid growth in culture. However, in place of E. coli, other unicellular microorganisms may be employed, for instance, fungi and algae. In addition, various forms of bacteria, such as salmonella or pneumococcus may be substituted for E. coli. Whatever host is chosen, it should not contain a restriction enzyme that would cleave the recombinant plasmid.

If E. coli is employed as a host, a preferable strain is MM294. Protocols for transformation of this particular host by a plasmid vector are well known, for instance, see Maniatis et al., supra at 255; and, Hanahan, 166 J. Mol. Biol. 557 (1983). Other strains of E. coli which also could serve as suitable hosts include RR1, DH1 (ATCC No. 33849) and C600. These strains and the MM294 strain are widely commercially available.

During transformation, only a small portion of the host cells are actually transformed, due to limited plasmid uptake by the cells. The cells that have been transformed can be identified by placing the cell culture on agar plates containing suitable growth medium and a phenotypic identifier, such as an antibiotic. Only those cells that have the proper resistance gene (e.g., to the antibiotic) will survive. If the recombinant pBR322 plasmid is used to transform E. coli strain MM294, transformed cells can be identified by using tetracycline as the phenotypic identifier.

Preparation of a Synthetic Oligonucleotide Screening Probe

A radiolabeled synthetic oligonucleotide corresponding to a portion of the amino acid sequence of the IL-2 receptor, as determined above, is used as a probe to screen the cDNA library. The hybridization of the synthetic oligonucleotide probe with plasmid cDNA prepared from the library clones is subsequently identified by autoradiography.

The amino terminal portion of the IL-2 receptor molecule has been identified and partially sequenced, above. A portion of this amino acid sequence, composed of the residues: Cys-Asp-Asp-Asp-Pro-Pro, is employed as the basis for the synthetic oligonucleotide probe. This particular portion of the amino acid sequence of the IL-2 receptor has the advantage of being short enough to be easily chemically synthesized, while also being long enough to be useful as a

direct probe for the IL-2 receptor gene. Also, this sequence corresponds to a particular codon composition that is relatively free of ambiguity.

Applicants have developed two synthetic oligonucleotides from the above amino acid sequence for use as probes to screen plasmid DNA thought to contain the IL-2 receptor genes. The probes are composed of the following two sequences each having 17 bases: 5' G-G-$\begin{matrix}T\\C\end{matrix}$-G-G-G-T-C-G-T-C-G-T-C-A-C-A 3'. The particular compositions of these probes were arrived at after conducting initial primer extension analysis which enabled applicants to eliminate other possible oligonucleotide sequences corresponding to the above-identified amino acid sequence. The compositions of the probes are the same except for the third nucleotide from the 5' end, which in one oligonucleotide is composed of thymidine and in the other is composed of cytosine. Also, the last nucleotide of Pro residue was not employed thereby to reduce the ambiguity of the oligonucleotide probes.

Although the described oligonucleotide sequences are the preferred composition of the synthetic probes of the present invention, it is to be understood that probes of other compositions corresponding to additional partial amino acid sequences of the IL-2 receptor molecule can be employed without departing from the spirit or scope of the present invention.

The synthetic oligonucleotide probes may be chemically synthesized by well-known techniques, such as by phosphodiester or triester methods. Methods for triester synthesis are set forth in Sood et al., 4 Nucl. Acid Res. 2557 (1977); and, Hirose et al., 28 Tet. Lett. 2449 (1978). After synthesis, the oligonucleotide probe is labeled with T4 polynucleotide kinase and ^{32}P-ATP, for instance by the protocol set forth in Maniatis et al., supra at 122. Advantageously, the oligonucleotide probes can be synthesized with OH 5' termini thereby avoiding the phosphatase procedure typically required.

Screening of cDNA Library

In the screening procedure of the present invention, the transformed bacteria cultures are pooled into groups. After the replicated plasmids have been extracted from the transformants, DNA is prepared by cleaving the plasmids at the Pvu II and Hind III restriction sites, both being unique sites on the hybrid plasmid. The resulting DNA segments are fractionated by electrophoresis on agarose gels and then directly analyzed by Southern blotting, for instance as described in 98 J. Mol. Biol. 503 (1975). The DNA that binds to the nitrocellulose filter in the Southern blotting procedure is hybridized with the labeled oligonucleotide probes. The specific DNA fragments that hybridize to the probe are identified by autoradiography.

The particular pool(s) of clones that give a signal following autoradiography are plated out and used in direct bacterial colony hybridization on a nitrocellulose filter with the same above-identified oligonucleotide probes. After completion of the hybridization, the nitrocellulose filter is monitored by autoradiography to identify positive colonies. In the present invention, applicants discovered two such positive colonies. Plasmid DNA, designated as IL-2 Rec N4 (hereinafter "N4") and IL-2 Rec N1 (hereinafter "N1") were prepared from the two particular positive colonies identified.

Characterization of Screened cDNA

The plasmid DNA prepared above is initially characterized by restriction enzyme mapping. Various strategies for restriction enzyme mapping are discussed by Maniatis et al., supra at 374. One preferred technique involves the partial digestion of end-labeled fragments of linear DNA. This technique, developed by Smith and Birnstiel, 3 Nucl. Acids Res. 2387 (1976), is now well known in the art. Partial restriction enzyme maps of the N4 cDNA clone in the region of the IL-2 receptor gene and of the N1 cDNA clone are shown in FIGURE 1. A distance scale for 100 nucleotide base pairs ("bp") is also shown. The Pst I sites shown in brackets are those generated by the cloning procedures.

The mapped plasmid cDNAs are initially partially sequenced to determine whether they are homologous to the amino acid sequence of the IL-2 receptor. Although applicants have ascertained that both cDNA clones illustrated have nucleotide sequences corresponding to the known N-terminus amino acid sequence of the IL-2 receptor, as discussed below, only the pN4 cDNA clone contains the gene coding for IL-2 receptor. The N-terminus of the mature IL-2 receptor protein is located at the Sst I site of the N4 clone.

Thereafter, the cloned cDNA's are sequenced using chain-termination method. This method of nucleotide sequencing was originated by Sanger et al., 70 Proc. Natl. Acad. Sci. (USA) 5463 (1977). See U.S. Patent No. 4,322,499. Methods for chain-termination sequence determination are set forth in the Amersham Handbook entitled, M13 Cloning and Sequencing, Blenheim Cresent, London (1983) (hereinafter "Amersham Handbook"); Messing, 2 Recombinant DNA Technical Bulletin, NIH Publication No. 79-99, 2, 43-48 (1979); Norrander et al., 26 Gene 101 (1983); Cerretti et al., 11 Nucl. Acids Res. 2599 (1983); and, Biggin et al., 80 Proc. Natl. Acad. Sci. (USA) 3963 (1983). M13 filamentous phage are employed as vectors to clone the DNA sequences of interest. These phage vectors provide single-stranded DNA templates which are readily sequenced by chain-termination method, which involves priming a single-stranded template molecule with a short primer strand

having a free 3' hydroxyl group and then using DNA polymerase to copy the template strand in a chain extension reaction using all four deoxyribonucleotide triphosphates, i.e., dATP, dCTP, dGTP, and dTTP (collectively referred to as "dNTPs"), with one of them being radiolabeled. In the synthesis reaction, a nucleotide specific chain terminator lacking a 3'-hydroxyl terminus, for instance, a 2', 3' dideoxynucleotide triphosphate ("ddNTP"), is used to produce a series of different length chain extensions. The terminator has a normal 5' terminus so that it can be incorporated into a growing DNA chain, but lacks a 3' hydroxyl terminus. Once the terminator has been integrated into the DNA chain, no further deoxynucleotide triphosphates can be added so that growth of the chain stops. Four separate synthesizing reactions are carried out, each having a ddNTP of one of the four nucleotide dNPTs, i.e., dATP, dCPT, dGTP and dTTP. One of the normal dNTPs is radiolabeled so that the synthesized strands after having been sorted by size on a polyacrylamide gel, can be autoradiographed. The chain extensions from the four reactions are placed side by side in separate gel lanes so that the pattern of the fragments from the autoradiography corresponds to the DNA sequence of the cloned DNA.

The DNA and corresponding amino acid sequences of the N4 and N1 clones from the 5' ends to the Xba I restriction site, as determined by the above techniques, are illustrated in FIGURE 2. As detailed below, the gene coding for IL-2 receptor is contained in the N4 clone and not in the N1 clone. In FIGURE 2, the nucleotide sequence shown is from the N4 clone except for the sequences upstream from the arrow, which are derived from the N1 clone. The arrow marks the 5' end of the insert in the N4 clone. The nucleotides are numbered from the position of the initiator methionine codon to the TAG termination codon. The amino acids are numbered beginning from the mature NH_2-terminus of the IL-2 receptor protein, i.e., the Glu residue, marked with a star, and extending to the Ile residue (251) located adjacent the termination codon TAG. The IL-2 receptor gene, extending from the initiator methionine codon to the TAG termination codon, is shown as a box portion in FIGURE 1. Correspondingly, the coding region of the N1 clone is shown as a box portion. The restriction enzyme cleaving sites identified in FIGURE 1 are also indicated in FIGURE 2.

The base sequence of the N1 clone differs from the N4 clone, in that the N4 clone contains a 216 base pair insert sequence not present in the N1 clone, extending from nucleotides 370 to 585 (underlined in dots in FIGURE 2). This 216 base pair insert is shown in FIGURE 1 as the unshaded box portion of the N4 clone. The two clones also differ at nucleotides 148, 183, 322 and 327.

In addition, as shown in FIGURE 2, three of these sequence differentials would cause amino acid changes. In terms of similarities, both clones contain the sequence of the oligonucleotide probe employed above, with a single base pair mismatch, and both encode the amino acid sequence determined above for the NH_2-terminus of the IL-2 receptor. Both also encode a stretch of 15 amino acids immediately preceding the NH_2-terminus sequence, which starts with a methionine residue and has many of the characteristics of a hydrophobic signal peptide expected from membrane or secreted proteins.

In preparation for the sequencing procedures, the cDNA clones shown in FIGURE 1 are digested with various restriction endonucleases in various combinations and then the resulting DNA fragments cloned into M13 phage vectors to form single stranded DNA templates. A universal primer is used to sequence the sense and nonsense strands. Rather than relying on the sequencing results obtained from sequencing the entire length of the fragments with a single chain termination procedure, in the longer fragments additional synthetically produced primers are used to initiate the chain termination procedure from intermediate locations along the lengths of the fragments. By this process, both strands of the cDNA clones shown in FIGURE 1 are sequenced in overlapping fashion, thereby serving to redundantly confirm the sequences.

It is to be understood that rather than employing the chain-termination technique outlined above, other known methods may be utilized to sequence the IL-2 receptor gene without departing from the spirit or scope of the present invention. For instance, the chemical degradation method of Maxam and Gilbert as set forth in 74 Proc. Nat'l Acad. Sci. (USA) 560 (1977) can be used.

Expression Of Functional IL-2 Receptor From cDNA Clones

To determine whether the cDNA coding regions of the N1 or N4 clones could encode a functional IL-2 receptor, the clones are expressed in mammalian cells. Hybrid cDNA fragments containing the coding regions of the N4 and N1 clones are inserted into a plasmid vector derived in part from simian virus 40 ("SV40"). The genome of this virus consists of a single, small, covalently closed circular DNA molecule whose entire nucleotide sequence has been determined, Fiers et al., 237 Nature, (London) 113-120 (1978), and Reddy et al., 200 Science 494-502 (1978). The two constructed vectors, designated as pMLSV-N1/N4-S and pMLSV-N1/N4-X, having the coding regions of the N4 and N1 clones, respectively, are illustrated in FIGURE 3A.

The above-delineated vectors are transfected into mammalian cells. After subsequent incubation, the cells are harvested and assayed for expression of mature IL-2 receptor by their ability to bind to labeled IL-2 or the

labeled 2A3-A1H monoclonal antibody directed against the IL-2 receptor. Labeled 2A3-A1H monoclonal antibody may be prepared as described above. IL-2 may be prepared by established methods, such as set forth in U.S. Patent No. 4,401,756, and in Urdal et al., 296 J. Chromatog. 171 (1984) and then radiolabeled, for instance by use of a radioiodination reagent such as Enzymobead ^{R} (BioRad Laboratories, Richmond, CA.). As shown in sections B and C of FIGURE 3, the mammalian cells transfected with the pN1/N4-S vector specifically bound to both IL-2 and the 2A3-A1H monoclonal antibody. However, neither pN1/N4-X or mock-transfected cells (prepared as a control) specifically bound to IL-2 or the 2A3 monoclonal antibody. Since the pN1/N4-S vector contained the coding region of the N4 clone, this indicated that this clone contains the gene coding for the functional IL-2 receptor protein, whereas the N1 clone does not.

The processes and products of the present invention are further illustrated by the following examples.

EXAMPLE 1

Preparation of IL-2 Receptor Containing Extracts From Malignant Cell Line

Hut-102 cells in a concentration of 2×10^5 cells per ml were cultured in 100-500 ml volumes in various plastic culture flasks or bottles (Falcon Plastics, Oxnard, CA) in RPMI-1640 medium. The medium was supplemented with 10% FCS, 2 mM glutamine, 100 U/ml penicillin and 100 ug/ml streptomycin. Since the HUT-102 cells have been reported to produce human T-cell leukemia virus (HTLV-1), work with this cell line was performed in a P-3 isolation facility.

The cells were cultured for 3-5 days in a humidified atmosphere of 5% CO_2 in air. After this period of time, viable cells were harvested by centrifugation and washed three times in PBS. Thereafter, the cell pellet was suspended in a volume that is three times the volume of the cell pellet in a solution composed of PBS containing 1% (w/v) Triton X-100 detergent and 2 mM PMSF. This mixture was kept on ice and periodically vortexed for 30 minutes. The extract was then centrifuged at 20,000 x g for 20 minutes to remove nuclei and insoluable debris. The cell extract, as thus prepared, was then stored at -70°C until used.

EXAMPLE 2

Preparation of IL-2 Receptor Containing Extracts From Lectin Activated Normal Cells

Human peripheral blood mononuclear cells were prepared by Ficoll-Hypaque density gradient centrifugation as described by Boyum, supra. The resulting cells were incubated separately in 100-mm plastic petri dishes in 8% FCS at a concentration of 2-5 x 10^6 per ml. The adherent cells were recovered with a rubber policeman after removing nonadherent cells with three media washes. The E^- adherent cells together with the E^+ nonadherent cells in a ratio of 1:25 were placed in bulk culture in 75-cm^2 flasks at a concentration of about 1-2 x 10^6 cells/ml in RPMI-1640 medium supplemented with 10% FCS, 100 U/ml penicillin and 100 ug/ml streptomycin. Activation was accomplished with 1% (v/v) PHA (Difco Laboratories, Detroit, MI). The cultures were incubated at 37°C in an humified atmosphere of 5% CO_2 in air. Aliquots containing approximately 1-2 x 10^7 cells were removed at various times for analysis of cell surface IL-2 receptors.

Cells were harvested by centrifugation approximately 72 hours after mitogen stimulation, and washed three times with PBS. The resulting cell pellet was suspended in a volume three times the volume of the pellet in a solution composed of PBS containing 1% (w/v) Triton X-100 detergent and 2 mM PMSF. The resulting mixture was kept on ice with periodic vortexing for 30 minutes. Thereafter, the extract was centrifuged at 20,000 x g for 20 minutes to remove nuclei and insoluable debris. The resulting cell extracts were stored at -70°C centrigrade until used.

EXAMPLE 3

Production of Monoclonal Antibody To IL-2 Receptor

Female BALB/c (Jackson Laboratories, Bar Harbor, ME) of ages of from 8-12 weeks were immunized intradermally in the hind legs with 10^7 PHA/PBL. Prior to immunization, the PHA/PBL cells were prepared as an emulsion by mixing these cells with 0.4 ml of complete Freund's adjuvant (Difco Laboratories). After the initial immunization, the mice were rechallenged weekly for four weeks with 10^7 PHA/PBA in incomplete Freund's adjuvant.

Periodically, serum from the mice was collected and tested individually for binding to PHA/PBL by ELISA, in a manner well known in the art. The animals found to have the highest response were given an additional intravenous injection of 10^7 PHA/PBL in PBS. Four days later, the animals were sacrificed by cervical dislocation. The spleens of the animals were harvested

and single cell suspensions prepared therefrom. The spleen cells were cultured in medium.

Fusion was achieved by mixing approximately 20×10^6 spleen cells with approximately 10×10^6 NS-1 murine myeloma cells in a 50 ml conical centrifuge tube. The cell mixture was pelleted by centrifugation for 5 minutes at 200 x g, and then the supernate removed by aspiration. The centrifuge tube with its intact cell pellet was transferred into a 37°C water bath. Then polyethylene glycol 15 w (Eastman, Inc.) (50% (w/v) in RPMI-1640 was added to the cell pellet in dropwise manner at a ratio of 1 ml of PEG/1.6×10^8 spleen cells. Thereafter, one volume of RPMI-1640 and 10 volumes RPMI 1640 containing 15% FCS and 1 mM pyruvate were slowly added during gentle stirring. Then, the cell suspension was centrifuged at 200 x g for 5 minutes and the supernate discarded to complete the fusion process.

The hybrid cells were selected by resuspending the resulting cell pellet in Click's medium containing 15% FCS and 100 mM sodium pyrvate. The unfused myeloma driver cells (NS-1), double NS-1 hybrids, unfused spleen cells and double spleen cell hybrids were prevented from proliferation by the addition to the medium of approximately 13.6 mg/L of hypoxanthane, 0.176 mg/L aminopterin and 3.88 mg/L of thymidine. The suspension was then divided into 200 ul aliquots in flat-bottom microliter plates (No. 3596, Costar Inc., Cambridge, MA). The cultures were maintained at approximately 37° in a humified atmosphere of 5% CO_2 in air.

After 10 days of culture, a 100 ul aliquot of supernate was removed from each viable culture and tested in an ELISA assay for binding to PHA/PBL (IL-2 receptor positive) or PBL (IL-2 receptor negative). Hybrids which demonstrate significant binding to PHA/PBL and little or no binding to PBL were transferred to 1 ml cultures and gradually weaned to HAT-free media. These hybrids were subcloned by limiting dilution cultures. Through this process, applicants have identified one particular hybrid clone, designated as 2A3-A1H, which significantly inhibits both mitogen and antigen induced proliferation of human PBL. Samples of this cell line are on deposit with the American Type Culture Collection ("ATCC"), Rockville, MD., under accession No. HB 8555. The 2A3-A1H monoclonal antibody has been characterized as of the $_1$K isotype that exhibits a very high affinity to the human IL-2 receptor. This antibody inhibits the binding of IL-2 to its receptor and is antagonistic of IL-2 action.

EXAMPLE 4

In Vivo Production of Hybridoma Cells Producing Monoclonal Anti-IL-2 Receptor Antibodies

Anti-IL-2 receptor antibody was produced in high concentration in vivo by intraperitoneal injection of BALB/c mice with approximately 1-10 x 10^6 hybridoma cells. One week prior to hybridoma cell injection, recipient BALB/c mice were given approximately 1.0 ml of pristane intraperitoneally as an ascites inducing irritant. From 8 to 14 days after hybridoma injection, intraperitoneal ascites were collected and each volume of fluid is mixed with 0.9 volume of 45% saturated ammonium sulfate and stirred overnight. The precipitate was separated by centrifugation and redissolved in phosphate buffer (0.05M), pH 6.8. Residual ammonium sulfate was removed by dialysis against the same buffer.

The protein solution was then passed over a 5 ml bed volume DE-52 column (Whatman, Clifton, NJ) and the fronting peak of protein was pooled. The pooled fractions were dialyzed against 0.02 M sodium borate, 0.1 M sodium NaCl, pH 8.5, ("BBS") and then applied to a 2.6 x 90 cm ACA-34 (LKB, Bromma, Sweden) gel filtration column previously equilibrated in the same buffer. The fractions corresponding to IgG were collected and pooled. Yields typically were in the range of 3 mg IgG/ml of ascites.

EXAMPLE 5

Purification of IL-2 Receptor By Affinity Chromotography

Cell extracts from normal and malignant cells produced by the procedures of Examples 2 and 3 were concentrated by affinity chromatography technique employing an initial gel column having control antibody for removing protein that might nonspecifically bind to mouse IgG and a second column having 2A3-A1H antibody bound thereto. The control antibody used in the initial column was secreted by the myeloma cell line MOPC-21. This antibody is of the same isotype as the 2A3-A1H antibody and is readily available.

To prepare the columns, purified 2A3-A1H and MOPC-21 antibodies were coupled to Affi-gel-10 (BioRad, Richmond, CA) according to the manufacturer's instructions. Equal volumes of moist Affi-gel-10 and antibody (3-5 mg/ml) in PBS were mixed together and incubated overnight at 4°C. Thereafter, unreacted sites on the Affi-gel-10 were blocked by addition of 100 ul of 1M glycine ethyl ester per ml of gel. Applicants found that the antibody-coupled gel routinely contained from 3 to 4 mg of antibody per ml of gel.

Because the 2A3-A1H antibody exhibits such an extremely high affinity for the IL-2 receptor, the receptor yield from the chromatography columns was improved by employing columns prepared with a mixture of MOPC-21 and 2A3-A1H antibody. A total of 3 to 4 mg IgG was still coupled per ml of gel, but only 10-30% of the IgG is composed of 2A3-A1H. The column having both MOPC-21 and 2A3-A1H antibody bound thereto will be referred to as the "2A3-A1H" column.

Prior to use, each gel was washed extensively with PBS and RIPA buffer. The MOPC-21 and 2A3-A1H gel columns were poured in 3 ml syringes that have their open ends closed with a cork and tubing, thereby to enable the columns to be run in either direction. The cell extracts, as prepared in Examples 1 and 2 above, were first applied to the MOPC-21 column at a flow rate of 0.1 ml/min at 4 °C to remove proteins that nonspecifically bind to the mouse IgG. This absorption was repeated once more and then the flow-through from the MOPC-21 column is twice applied to the 2A3-A1H column.

The 2A3-A1H column was then washed with 10 column volumes of PBS-1 % Triton X-100, 10 column volumes of RIPA buffer and lastly, 10 column volumes of PBS-1 % Triton X-100. Thereafter, the receptor was eluted from the column with 6M guanidine hydrochloride ("GuHCl") and 0.5 % Triton X-100. Eluate fractions in 1.2 ml volume were collected and each fraction was dialyzed against 3 M GuHCl in 0.5 % Triton X-100 for four hours. This was followed by dialysis against 1.5 M GuHCl in 0.5 % Triton X-100. Final dialysis was performed against PBS containing 0.5 % Triton X-100. Aliquots at each stage of the purification were saved for analysis of: biological activity by the above-described soluble receptor assays; protein concentration by fluorescamine assay with bovine serum albumin as a standard, as is well known in the art; and, protein heterogeneity by polyacrylamide gel electrophoresis with the protein being detected by silver staining, as also described above. From these assays, the IL-2 receptor from the HUT-102 cells was found to have a specific activity of approximately 2,000 fm receptor/ug protein. The specific activity from the PHA-PBL cells was somewhat less.

EXAMPLE 6

Reversed Phase High Performance Liquid Chromatography

The active fractions obtained in Example 5 were pooled for use as the starting material for the HPLC process. These fractions were pumped directly onto a 3.9 mm times 15 cm Vydac C-4 column, which had been previously equilibrated with 0.1 percent TFA in water, at a flow rate of about

1 ml/min with a Waters M-45 A solvent pump (Waters Associates, Millford, Maine). The loaded column was initially washed with 0.1% TFA to remove non-bound components until the absorbence at 214 nanometers as detected with a Waters Model 441 absorbence detector drops to base line. Elution of bound proteins was accomplished with a linear gradiant of 0-95% acetonitrile in 0.1 percent TFA (v/v) at a rate of 1% per minute. The IL-2 receptor protein was found to elute off the column in the 50 to 55% acetonitrile fractions.

One minute fractions were collected (1 ml) and 50 ul aliquots were removed from each fraction for analysis by polyacrylamide gel electrophoresis followed by silver staining. This technique resulted in the separation of a single band of protein at a molecular weight of 55,000 daltons for the HUT-102 receptor molecule. The PHA-PBL receptor molecule, which eluted at the same position on the HPLC as the HUT-102 receptor molecule, exhibited a single band of protein having a molecular weight of 60,000 daltons.

Aliquots in 50 ul volumes were also removed from the minute fractions for biological assay. The aliquots were dried under vacuum in the presence of 50 ug BSA. The dried residue was dissolved in PBS-2% Triton X-100 for analysis by the soluble receptor assay techniques discussed above. This assay indicated that the IL-2 from HUT-102 receptor had been purified from 1.26 fmole receptor/ug in protein the cell lysate starting material to approximately 21,000 fmole receptor/ug protein after the HPLC purification step. This equates to an increase in purification of the IL-2 receptor of about 16,670 times. The specific activity of the PHA-BPL receptor after the HPLC purification step was found to be approximately 5,000 fmole receptor/ug protein. It is clear from the single protein bands which resulted from the polyacrylamide gel electrophoresis and silver staining of the fractions collected after HPLC, and also from the specific activities of the fractions analyzed by the soluble receptor assays, essential homogeneity of the IL-2 receptor molecule was achieved.

EXAMPLE 7

Protein Sequencing

Purified IL-2 receptor from Example 6 was dried under vacuum to a final volume of approximately 100 ul and then subjected to automated amino terminal Edman degration using an Applied Biosystems Model 470A protein sequencer. Fractions from the sequencing cycles were evaporated to dryness and then resuspended in acetonitrile/H_2O (50:50) before injection into an HPLC column for residue identification.

By the above process, the amino-terminal amino acid sequence for the IL-2 receptor from both the HUT-102 and PHA-PBL cells were found to be the same. The first 15 residues of the N-terminal portion of the IL-2 receptor molecule was determined to be composed of the following sequence: Glu-Leu-Cys-Asp-Asp-Asp-Pro-Pro-Glu-Ile-Pro-His-Ala-Thr-Phe. This amino acid sequence was compared with known protein sequences contained in the National Biomedical Research Foundation protein data base "SEARCH" (January, 1984), and was not significantly homologous to any protein sequence contained in this data base.

EXAMPLE 8

Preparation of Polyadenylated mRNA

Hut-102 cells at a concentration of approximately 2×10^5 cells/ml were cultured in 100-500 ml volumes in RPMI-1640 medium supplemented with 10% FCS (v/v), 2 mM glutamine, 100 U/ml penicillin and 100 ug/ml streptomycin. The cells were cultured for 3-5 days in a humidified atmosphere of 5% CO_2 in air. After this period of time, viable cells were harvested by centrifugation.

Total RNA was extracted from the Hut-102 cells by the method as decribed by Chirgwin et al., supra. In this procedure guanidinium thiocyanate was used to denature the cellular protein including the RNase at a rate that exceeds the rate of RNA hydrolysis by RNase. The mRNA was removed from the cellular protein by ultracentrifugation through a dense cushion of cesium chloride.

Thereafter, polyadenylated mRNA was separated from the extracted protein on an oligo (dT)-cellulose chromatography column using the method disclosed by Maniatis et al., supra at 197. Briefly, the column was prepared with application buffer composed of 20 mM Tris-Cl (pH 7.6), 0.5 M NaCl, 1 mM ethylene diamine tetraacetate ("EDTA") and 0.1% sodium dodecyl sulfate ("SDS"). The pellet was dissolved in water and application buffer and then loaded onto the column. The nonadsorbed material was removed by initial washings with application buffer followed by additional washings with application buffer containing 0.1 M NaCl. The retained polyadenylated mRNA was removed with buffers of reduced ionic strength composed of 10 mM Tris-Cl (pH 7.5), 1 mM EDTA and 0.05% SDS. The eluted polyadenylated mRNA was precipitated at -20°C with 1/10 volume sodium acetate (3M, pH 5.2) and 2.2 volumes of ethanol. After elution of the polyadenylated mRNA from the oligo (dT)-cellulose column, the integrity of the polyadenylated mRNA was confirmed

by electrophoresis through agarose gels as detailed in Maniatis et al., supra at 199.

EXAMPLE 9

Construction of cDNA Library

A library of double-stranded cDNA corresponding to the mRNA was prepared from the purified mRNA in Example 8 by employing the procedure detailed by Maniatis et al., supra at 229. Oligo-dT was hybridized to the polyadenylated tail of the mRNA to serve as the primer for the reverse transcription of the first cDNA strand. The enzyme avian myeloblastosis virus ("AMV") reverse transcriptase was employed to synthesize the first DNA strand by using the mRNA as a template. This procedure resulted in a hairpin loop being formed at the 3' end of the initial cDNA strand. The hairpin loop served as an integral primer for the second cDNA strand. After the mRNA strand was degraded with NaOH, the second cDNA strand was synthesized with DNA polymerase I. The hairpin was then removed with nuclease S1 to produce double-stranded cDNA molecules.

The double-stranded cDNA was fractionated into size classes by Sephacryl S-400 column chromatography and monitored by alkaline agarose electrophoresis using end-labeled fragments of pBR322 DNA as molecular-weight markers. Strands having a length of less than 500 bp were culled out to avoid needless cloning of these undersized cDNA fractions.

The double-stranded cDNA fractions, as prepared above, were inserted into the Pst I site of the pBR322 plasmid. The double-stranded cDNA was tailed with poly (dC) at its 3' ends. The plasmid pBR322 was digested with Pst I endonuclease and then tailed with poly (dG) at its 3' ends. The tailed plasmid DNA and the tailed cDNA were annealed in annealing buffer (0.1 M NaCl, 10 mM Tris-Cl (pH 7.8) and 10 mM ETDA) to form novel recombinant plasmids.

The recombinant plasmids were transformed into E. coli strain MM294 by using the procedure of Hanahan, supra in which the E. coli cells were prepared by growth in elevated levels of Mg^{2+}. The transformation hosts were plated and then transformants are identified by use of tetracycline as a phenotypic identifier. By use of this technique, applicants obtained approximately 2×10^6 independent transformants.

EXAMPLE 10

Preparation of Synthetic Oligonucleotide Screening Probes

Synthetic oligonucleotides were employed as a probe in screening the cDNA library prepared as set forth above in Example 9. The probes were composed of the following compositions: 5' G-G-$\begin{matrix}T\\C\end{matrix}$-G-G-G-T-C-G-T-C-G-T-C-A-C-A 3'. These oligonucleotide probes were chemically synthesized by triester method using the well known techniques of Sood et al., supra and Hirose et al., supra.

After chemical synthesis had been completed, the 5' ends of the oligonucleotide probes were labeled with ^{32}P. To facilitate labeling, the 5' ends of the oligonucleotide were synthesized with OH termini, thereby eliminating the phosphatese treatment which typically must be employed when labeling DNA fragments. The labeling protocol included adding 1 ul of the synthetic oligonucleotides to 16 ul of ^{32} P - ATP (7000 Ci/mM), 1 microliter ("ul") (10 U) of T4 polynucleotide kinase and 2 ul of 10 x kinase buffer I. The 10 x kinase buffer I was composed of 0.5 M Tris-Cl (pH 7.6), 0.1 M $MgCl_2$, 50 mM dithiothreitol, 1 mM spermidine and 1 mM ETDA. The reaction was carried out at 37°C for 30 minutes, and thereafter the synthesized oligonucleotides were extracted with phenol/chloroform. The labeled probes were separated from unlabeled oligonucleotides by chromatography on or centrifugation through Sephadex G-50 columns.

EXAMPLE 11

Screening of cDNA Library

To facilitate initial screening of the cDNA library prepared in Example 9 above, the transformed bacteria cultures were grouped into pools each having approximately 5,000 different clones. Plasmid DNA was removed from samples of the host bacteria by the well known alkaline lysis method, for instance as described by Ish-Horowicz and Burke, 9 Nucl. Acids Res., 2989 (1981).

The isolated plasmids were separated into two fragments. This was accomplished by initially digesting the plasmids to completion with Pvu II and Hind III. The plasmids were redissolved in 20 ul of 1 x Hind III buffer (7 mM Tris, (pH 7.4), 7 mM magnesium chloride, 60 mM NaCl) and then 1 ul of Pvu II and 1 ul of Hind III restriction endonucleases were added. This mixture was incubated at 37°C for two hours.

Next, the plasmid digests were fractionated by electrophoresis through 0.8% agarose gel with markers of appropriate size. The agarose gel was

blotted onto nitrocellulose filter using the well known method described by Southern, supra. After the transfer process, the filter was air dried and baked for two hours at approximately 80°C under a vacuum to bind the DNA fragments to the nitrocellulose.

The bound DNA was next hybridized with the labeled oligonucleotide probes. Briefly, the baked nitrocellulose was presoaked in 6 x saline sodium citrate ("SSC") (20 X SSC is composed of 175.3g of NaCl and 88.2g of sodium citrate in 800 ml of H_2O, with pH adjusted to 7.0 with 10N NaOH) and then incubated at 50°C for 2-4 hours in prehybridization buffer composed of 6 x SSC, 0.5% NP40 detergent, 0.1% sarcosyl, 5 x Denhardt's solution (0.02% Ficoll, 0.02% polyvinyl pyrrolidone, 0.02% BSA) and 100 ug/ml denatured salmon sperm DNA (Sigma Type III, sodium salt). The filter was then incubated overnight at 50°C with the ^{32}P-labeled oligonucleotide probe (10^6 cpm/ul) (from Example 10) in hybridizing solution as above. After overnight hybridization, the filter was washed extensively with 6 x SSC at room temperature and then for 5 minutes at 50°C with 6 x SSC. After air drying, the filter was subjected to autoradiography at - 70°C.

From the autoradiography, applicants found several pools of transformants generating hybridizing bands. The appropriate pools of the transformants were plated out and then used in direct bacterial colony hybridization on nitrocellulose paper with the labeled oligonucleotide probe under the same hybridizing conditions as above. By this process, two positive colonies were identified.

EXAMPLE 12

Restriction Enzyme Mapping of Screened cDNA

Plasmids, designated as N4 and N1, were prepared from the identified positive colony by the procedures set forth in Example 9. Samples of the N4 and N1 plasmids transformed into E. coli strain MM294 are on deposit with the ATCC, under Accession Nos. 39752 and 39751, respectively. Thereafter, the N4 and N1 plasmids were analyzed by restriction enzyme mapping using the standard method developed by Smith and Birnstiel, supra, involving partial digestion of end-labeled fragments of the linearized DNA. The DNA fragments were labeled at their 5' termini with ^{32}P-phosphoryl groups using polynucleotide kinase and ^{32}P - ATP. The labeled DNA strands were then cleaved asymmetrically with a suitable restriction enzyme to provide two fragments, each labeled at only one of its ends. These labeled fragments were isolated by gel electrophoresis. Each of the two fragments was partially digested by appropriate restriction enzymes.

Although a large spectrum of digestion fragments were produced, the labeled fragments formed a simple overlapping series each having a common labeled terminus. These fragments were fractionated by gel electrophoresis and then examined by autoradiography. The locations of the fragments on the gel correspond directly to the order of the restriction sites along the plasmid DNA.

By this procedure, applicants partially mapped the restriction sites, as shown in FIGURE 1, of the N4 and N1 plasmid cDNAs in the region of the IL-2 receptor gene.

EXAMPLE 13

Sequencing of Screened cDNA

The DNA fragments shown in FIGURE 1 were initially partially sequenced by the dideoxy chain termination method. From the sequencing results, applicants confirmed that the N4 DNA fragment shown in FIGURE 1 contains the gene coding for the IL-2 receptor, with the N-terminus of the mature IL-2 receptor protein being located at the Sst I site of the DNA fragment shown in FIGURE 1.

Thereafter, the portions of the N4 and N1 clones from the 5' terminals to the Xba I restriction sites were sequenced by the chain termination protocol essentially as described in the Amersham Handbook, supra, with the variations set forth below. The N4 and N1 clones were digested with Pst I, Sst I and Xba I restriction endonucleases in various combinations and then the resulting DNA fragments were cloned into strains mp18 and mp19 of the M13 single-stranded filamentous phage vector (Amersham, Arlington Heights, Illinois). The mp18 and mp19 phage vectors, as set forth in Norrander et al. supra, contain the following unique cloning sites: Hind III; Sph I; Pst I; Sal I; Acc I; Hinc II; Xba I; BamHI; Xma I; Sma I; Kpn I; Sst I; and, EcoRI. The composition of the mp18 and mp19 vectors are identical, with the exception that the order of the above-identified restriction sites are reversed in the mp19 vector so that both strands of a DNA segment may be conveniently sequenced with the two vectors. The mp18 and mp19 vectors, with fragments of the N4 and N1 clones inserted therein, were used to transform E. coli JM103 and JM105 of the strain K12 (Bethesda Research Laboratories, Bethesda, Maryland) to produce relicate single-stranded DNA templates containing single-stranded inserts of the sense and antisense strands.

The synthetic universal primer: 5'-CCCAGTCACGACGTT-3' (P-L Biochemicals, Milwaukie, Wisconsin), was annealed to the single-strand DNA templates and used to prime DNA synthesis as described above at page 23. Thereafter, the extension fragments were size-separated by gel electrophoresis and autoradiographed from which the nucleotide sequences of the fragments were deduced.

An additional primer was employed to prime synthesis from an intermediate location along the sense strands of the N4 and N1 clones. A primer having the composition: 5'-GTGACACCTCAACCTGA-3', corresponds to nucleotides 262 through 278 (FIGURE 2). The composition of this primer strand was established from the sequencing information previously obtained by the sequencing of the N4 and N1 clones from their 5' termini with the universal primer. An additional synthetic primer of the composition: 5'-TGTGACGAGGCAGGAAG-3' (corresponding to nucleotides 613 through 629 in FIGURE 2) was used in sequencing the antisense strands between the Xba I and Sst I sites of the N4 and N1 clones. By the above "walk down" method, the strands of the N4 and N1 clones were sequenced from their 5' terminals to their Xba I sites in an overlapping, redundant manner thereby confirming the nucleotide sequence of these clones. It is to be understood that other synthetic primers could have been employed to initiate chain extensions from other locations along the N4 and N1 clones, without departing from the scope of the present invention.

Deoxyadenosine 5' (alpha-[^{35}S] thio) triphosphate (hereinafter "dATP [alpha-^{35}S]") was used as the radioactive label in the dideoxy sequencing reactions. Also, rather than using the gel set forth at page 36 of the Amersham Handbook, a 6% polyacrylamide gel was employed (6% polyacrylmide gel, 0.4 mm thick, containing 7 M, urea 100 mM Tris borate (pH 8.1), and 2 mM EDTA).

As noted above, the nucleotide sequences of the N4 and N1 clones from their 5' terminals to the Xba I sites are illustrated in FIGURE 2. This segment of DNA was found to include the coding regions of the clones. The nucleotides are numbered from the position of the initiator methionine codon. The corresponding amino acids, as determined by the nucleotide sequence and by protein sequence analysis, are set forth above the appropriate codons. The amino acid composition of the IL-2 receptor gene extends from the mature NH_2-terminus of the IL-2 receptor molecule, i.e., the Glu residue, as marked with a star in FIGURE 2 (from which the numbering of the amino acid residues begins), to the Ile residue (No. 251) immediately preceding the termination codon TAG. Various restriction enzyme cleaving sites are also indicated in FIGURE 2. The portions of the coding regions of the N4 and N1 clones in FIGURE 2 are

illustrated as boxed regions in FIGURE 1, with the solid box portions indicating substantially corresponding portions of the clones and the open box portion depicting the 216 base pair sequence only present in the N4 clone.

EXAMPLE 14

Expression of Mature IL-2 Receptor In Mammalian Cells

The coding regions of the N4 and N1 clones were inserted into a plasmid vector for transfection of mammalian cells to ascertain whether either coding region encodes a functional IL-2 receptor. The transfected cells were assayed for expression of IL-2 receptor by their ability to bind either labeled IL-2 or a labeled monoclonal antibody directed against the IL-2 receptor, i.e., 2A3-A1H monoclonal antibody. Hybrid cDNAs containing the coding regions of the N4 and N1 clones (illustrated in FIGURE 3A), designated as pN1/N4-S and pN1/N4-X, respectively, were inserted into the pMLSV phage vector, shown as a circle, to produce the plasmids pMLSV-N1/N4S and pMLSV-N1/N4X, respectively.

The pMLSV vector was derived principally from SV40 whose genome consists of a single, small covalently closed DNA molecule that has been entirely sequenced, Fiers et al., supra, and Reddy et al., supra. The pMLSV vector is composed of four parts, including the stippled box portion shown in FIGURE 3A which contains the control region of the SV40 plasmid (including the origin of DNA replication, enhancer elements and early and late promoters) (SV40 coordinates 5107-208). This vector portion was originally derived from the pSV2-dhfr vector as a Hind III-Pvu II fragment, Subramani et al., 1 Mol. Cell Biol. 854-864 (1981) and Lebowitz and Weissman, 87 Current Topics in Microbiology and Immunology 43 (1979). For use in the pMLSV plasmid, the Pvu II site was converted into a BamHI site and the Hind III site converted to Xba I site.

Downstream from the early promoter, the pMLSV vector includes a synthetic polylinker of the composition:

5'-CTAGAAGCTTGGTACCAGCTGCAGATCTC
3'-TTCGAACCATGGTCGACGTCTAGAG

GAGAATTCATCGAT-3'
CTCTTAAGTAGCTAGATC-5'.

This polylinker has Xba I cohesive termini and contains the following restriction sites: Hind III; Kpn I; Pvu II; Pst I; Bgl II; Xho I; EcoRI; Cla I; and, Xba I.

The hatched box portion of the plasmid contains the SV40 small t antigen donor and acceptor splice junctions (SV40 coordinates 4035-4656) and the SV40 polyadenylation signal (SV40 coordinates 2469-2706), originally derived from the pSV2-dhfr plasmid as a Bgl II-BamHI fragment, Subramani, supra. The Bgl II site was converted to a Xba I site for correspondence with the adjacent terminal of the synthetic polymer.

The long thin line portion of the pMLSV plasmid is derived from the plasmid pML2d, a derivative of plasmid pBR322, that lacks sequences inhibitory to DNA replication in mammalian cells, Sarver et al., 79 Proc. Natl. Acad. Sci. (USA) 7147-7151 (1982); and, Luskey and Botchan, 293 Nature 79-81 (1981).

Because it is known that the presence of dG-dC tails at the 5' end of a cDNA insert can inhibit its expression in mammalian cells (for instance, see Riedel et al., 3 EMBO Journal 1477 (1984)), hybrid cDNAs were constructed by combining portions of the N4 and N1 cDNA clones with the sequences derived from the N4 clone shown as open boxes and the sequences derived from the N1 clone shown as solid boxes in FIGURE 3A. As illustrated, the pN1/N4S hybrid fragment includes the portion of the N4 clone from the BamHI site to the 5' Sst I site to which is attached the 5' Pst I - Sst I fragment from the N1 clone, and thus contains the coding region of the N4 clone. The pN1/N4X hybrid cDNA contains a 5' Pst I-Xba I fragment from the N1 clone and a Xba I-BamHI fragment from the N4 clone, and thus contains the coding region of the N1 clone. It will be appreciated that both of the hybrid cDNAs take advantage of the "natural" Pst I site in the 5' prime noncoding region of the N1 clone that lacks tails. The pN1/N4-S and pN1/N4-X hybrid cDNAs having Pst I and BamHI cohesive ends were inserted into the Pst I and Bgl II sites of the pMLSV plasmid by standard techniques, for instance, as detailed in Maniatis et al., supra, to form plasmid vectors pMLSV-N1/N4-S and pMLSV-N1/N4-X, respectively. The pMLSV-N1/N4-S plasmid vector has been deposited with the ATCC under Accession No. 39890.

The plasmids as prepared above were transfected into COS-7 monkey kidney cells (ATCC, Rockville, MD) by standard techniques, for instance, by essentially using the procedures described by Lauthman and Magnusson, 11 Nucl. Acid Res. 1295 (1983). Monolayers of COS-7 cells (10^6 cells per 10 cm plate) were washed twice with Tris-buffered saline ("TBS") and exposed to 10 ug of hybrid pMLSV-pN1/N4-S or pMLSV-pN1/N4-X DNA per plate in 1 ml TBS containing 500 ug/ml DEAE-Dextran (molecular weight 5×10^5; Sigma Chemical Company, St. Louis, MO.) for 30 minutes at room temperature. The cells were washed once more with TBS and fed with growth medium (Dubecco's Modified

Eagle's Medium with 10% (v/v) fetal bovine serum) containing 100 uM chloroquine (St. Louis, MO.). After incubation for five hours at 37° C, the medium was replaced by growth medium without chloroquine. The cells were then incubated at 37° C for 48 hours, after which time they were harvested by scraping.

The transfected COS-7 cells were screened for IL-2 receptor expression by ascertaining the ability of the cells to bind to ^{125}I-labeled anti-IL-2 receptor antibody 2A3-A1H (FIGURE 3B) and also to ^{125}I-labeled IL-2 (FIGURE 3C). The 2A3-A1H monoclonal antibody was prepared and radiolabeled to a specific activity of 9.8×10^{14} cpm/mM, as described above.

Purified IL-2 was radiolabeled using the Enzymobead radioiodination reagent (BioRad Laboratories, Richmond, CA.) essentially by the manufacturer's specifications. Fifty ul aliquotes of IL-2 (5×10^6 units) in 65% acetonitrile and TFA (pH 2.1) were combined with 50 ul of 0.2 M sodium phosphate (pH 7.2) and then the acetonitrile evaporated under nitrogen. Fifty ul of Enzymobead reagent, 10 ul of ^{125}I (1 mCi) and 10 ug of 2.5% Beta-D-glucose (BioRad Laboratories, Richmond, CA.) were added and then the mixture incubated at 25° C for 10 minutes. Twenty ul of 25 mM sodium azide and 10 ul of sodium metabisulfite (5 mg/ml) were then added, and after 5 minutes of incubation at 25° C, iodinated IL-2 was separated from unbound ^{125}I by chromatography on a 2 ml column of Sephadex G-25 equilibrated in 0.05 M sodium phosphate (pH 7.2) containing 0.1% v/v BSA and eluted with this same buffer. Based on an initial biologic specific activity for IL-2 of 1×10^6 units/ug protein, the radiolabeled preparation had an estimated specific activity of 1×10^{15} cpm/mM.

The binding assays were performed as described in Dower et al., 132 J. Immunol. 751 (1984). COS-7 cells (1.2×10^6) were incubated with either 5×10^{-9} M ^{125}I-2A3-A1H monoclonal antibody or 1.3×10^{-8} M ^{125}-IL-2 in a final volume of 150 ul of binding medium for 30 minutes at 37° C. Nonspecific binding was measured in the presence of 1000-fold molar excess of unlabeled 2A3-A1H monoclonal antibody or 150-fold molar excess of unlabeled IL-2. Replicate 70 ul aliquots of the above incubation mixtures were centrifuged through phthalate oil to separate the ^{125}I bound to COS-7 cells from the unbound cells (^{125}I labeled IL-2 or 2A3-A1H).

The results of the ^{125}I binding assay are set forth in panels B and C of FIGURE 3. As shown, only the pMLSV-N1/N4-S transfected COS-7 cells bound to the labeled IL-2 and labeled 2A3-A1H monoclonal antibody. Neither the pMLSV-N1/N4-X transfected COS-7 cells or the mock-transfected COS-7 control cells showed any specific binding of IL-2 or the monoclonal anti-IL-2

receptor antibody. Since only the pMLSV-N1/N4-S hybrid contains the N4 coding region, the functional IL-2 receptor protein is encoded thereby.

As will be apparent to those skilled in the art to which the invention is addressed, the present invention may be carried out by using cell lines, culture media, culture media additives, culture conditions, assays, antibodies, purification restriction mapping and sequencing techniques, and chromatography columns other than those specifically discussed above without departing from the invention. The particular materials and processes described above are therefore to be considered as illustrative and not restrictive.

CLAIMS:

1. A homogenous receptor for interleukin 2 comprising a protein characterized as having a molecular weight of about 55,000 to 60,000 daltons and an N-terminal amino acid sequence as follows: Glu-Leu-Cys-Asp-Asp-Asp-Pro-Glu-Ile.

2. A homogenous receptor for interleukin 2 comprising an amino acid sequence extending from residue No. 1 to residue No. 251 inclusive in FIGURE 2.

3. A homogenous receptor for interleukin 2 as claimed in claim 2 and further comprising the following N-terminal amino acid sequence:Met-Try-Gly-Leu-Leu-Thr-Phe-Ile-Met-Val-Pro-Gly-Cys-Glu-Ala.

4. Substantially pure DNA extracted from mammalian cells capable of producing IL-2 receptor molecules and comprising the nucleic acid sequence extending from nucleic acid base No. 64 to nucleic acid base No. 816 inclusive in FIGURE 2.

5. DNA as claimed in claim 4 and further comprising the N-terminal nucleic acid sequence extending from nucleic acid base No. 1 to nucleic acid base No. 63 inclusive in FIGURE 2.

6. Substantially pure DNA encoding for interleukin 2 receptor and comprising the nucleotide sequence underlined by dots in FIGURE 2.

7. An amino acid chain encoded by the nucleotide sequence of DNA as claimed in any one of claims 4 to 6.

8. A process for producing a homogeneous receptor for interleukin 2, comprising:

(a) culturing cells capable of expressing interleukin 2 receptor molecules in culture medium;

(b) harvesting said cells from the culture medium;

(c) extracting the interleukin 2 receptor molecules from said cells; and

(d) purifying said interleukin 2 receptor molecules with a reversed phase, high-performance liquid chromatography column containing methyl, octyl or octyldecyl groups covalently bonded to silica gel, whereby the interleukin 2 receptor molecules are retained by the column.

9. A process as claimed in claim 8, wherein culturing said interleukin 2 receptor expressing molecules includes culturing T-cells in the presence of an activating agent.

10. A process as claimed in claim 8, wherein said interleukin 2 receptor expressing cells comprise malignant cells, e.g. T-lymphoma or T-leukemia cells, or normal T-cells.

11. A process as claimed in any one of claims 8 to 10, wherein said receptor molecules are initially partially purificd by passing the interleukin 2 containing extract through an affinity chromatography column prior to the reversed phase high-performance

liquid chromatography procedure, said column containing a gel substrate to which is bound an antibody that specifically binds to an antigenic epitope on the interleukin 2 receptor whereby the interleukin 2 receptor is retained by the chromatography column, eluting the retained interleukin 2 receptor from the column and pooling fractions exhibiting interleukin 2 activity.

12. A process as claimed in claim 11, wherein said antigenic epitope is included within the amino acid sequence extending from residue No. 1 to residue No. 10 inclusive in FIGURE 2, or is included within the amino acid sequence extending from residue No. 103 to residue No. 174 inclusive in FIGURE 2, or is included within the amino acid sequence extending from residue No. 1 to residue No. 251 inclusive in FIGURE 2.

13. A process for producing DNA encoding for interleukin 2 receptor comprising employing a hybridization probe having a base sequence corresponding to the amino acid sequence at the N-terminal end of interleukin 2 receptor to isolate a DNA fraction from a cDNA library prepared by the action of reverse transcriptase from polyadenylated mRNA extracted from a cell line or other source known to produce interleukin 2 receptor, rendering double-stranded, using DNA polymerase, said DNA fraction , incorporating the resulting DNA into a cloning vector, using the resulting vector to transform a host, and isolating from the resulting transformants, using a synthetic oligonucleotide probe, cloned vector DNA encoded for interleukin 2 receptor.

14. A process for producing interleukin 2 receptor which comprises cloning DNA which has been produced by a process as claimed in claim 13 or DNA as defined in any one of claims 4 to 6 in a mammalian cell system so as to express said DNA.


[PstI]
PstI
BamHI
100bp
XbaI
BglI
PstI
SstI
[PstI]
N4
[PstI]
XbaI
BglI
SstI
PstI
[PstI]
N1

FIG.1

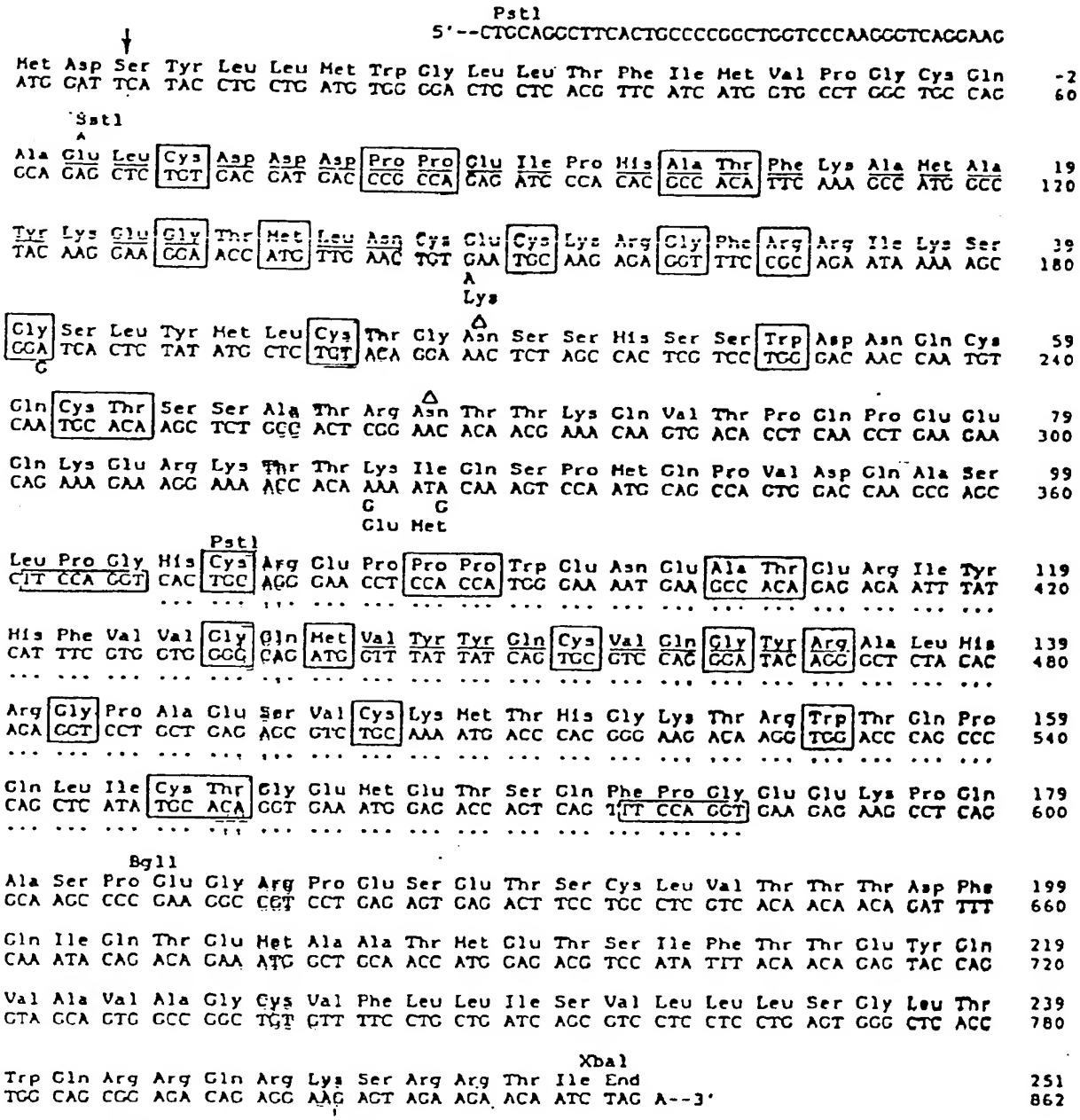
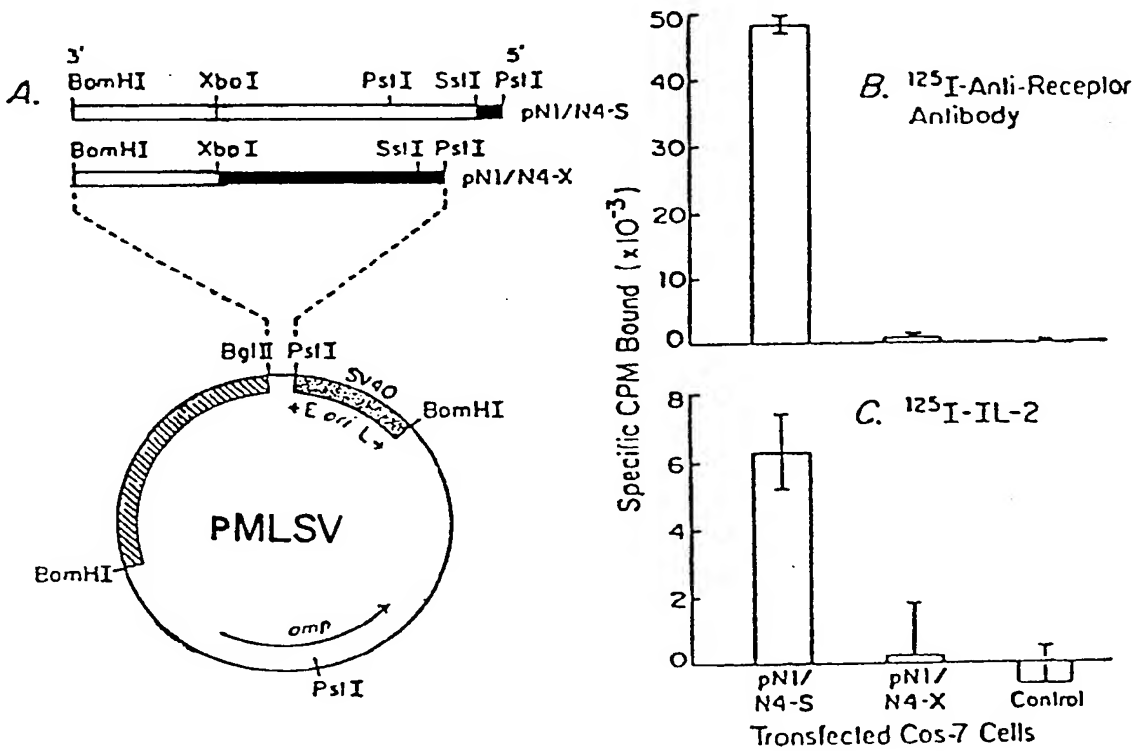
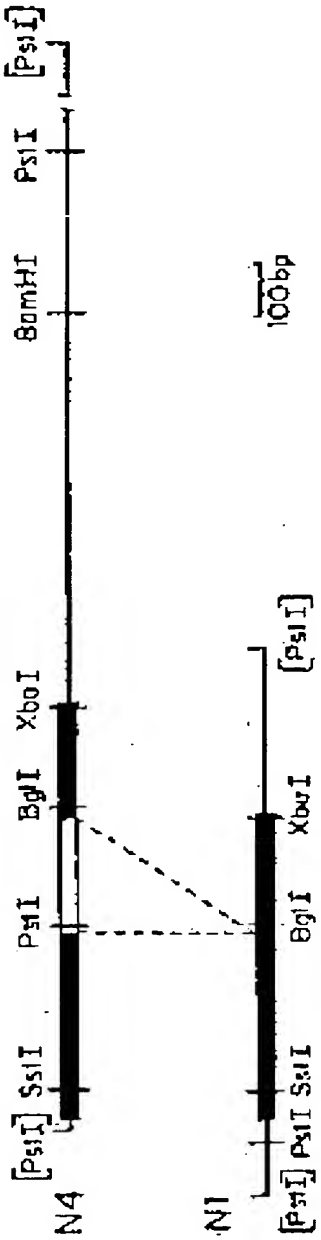

PstI
5'--CTGCAGGCTTCACTGCCCCGGCTGGTCCCAAGGGTCAGGAAG
Met Asp Ser Tyr Leu Leu Met Trp Gly Leu Leu Thr Phe Ile Met Val Pro Gly Cys Gln -2
ATG GAT TCA TAC CTG CTG ATG TGG GGA CTG CTC ACG TTC ATC ATG GTG CCT GGC TGC CAG 60
SstI
Ala Glu Leu Cys Asp Asp Asp Pro Pro Glu Ile Pro His Ala Thr Phe Lys Ala Met Ala 19
GCA GAG CTC TGT GAC GAT GAC CCG CCA GAG ATC CCA CAC GCC ACA TTC AAA GCC ATG GCC 120
Tyr Lys Glu Gly Thr Met Leu Asn Cys Glu Cys Lys Arg Gly Phe Arg Arg Ile Lys Ser 39
TAC AAG GAA GGA ACC ATG TTG AAC TGT GAA TGC AAG AGA GGT TTC CGC AGA ATA AAA AGC 180
A
Lys
Gly Ser Leu Tyr Met Leu Cys Thr Gly Asn Ser Ser His Ser Ser Trp Asp Asn Gln Cys 59
GGA TCA CTC TAT ATG CTC TGT ACA GGA AAC TCT AGC CAC TCG TCC TGG GAC AAC CAA TGT 240
C
Δ
Gln Cys Thr Ser Ser Ala Thr Arg Asn Thr Thr Lys Gln Val Thr Pro Gln Pro Glu Glu 79
CAA TGC ACA AGC TCT GCC ACT CGG AAC ACA ACG AAA CAA GTG ACA CCT CAA CCT GAA GAA 300
Gln Lys Glu Arg Lys Thr Thr Lys Ile Gln Ser Pro Met Gln Pro Val Asp Gln Ala Ser 99
CAG AAA GAA ACG AAA ACC ACA AAA ATA CAA AGT CCA ATG CAG CCA GTC GAC CAA GCC AGC 360
G G
Glu Met
PstI
Leu Pro Gly His Cys Arg Glu Pro Pro Pro Trp Glu Asn Glu Ala Thr Glu Arg Ile Tyr 119
CTT CCA GGT CAC TGC AGG GAA CCT CCA CCA TGG GAA AAT GAA GCC ACA GAG AGA ATT TAT 420
His Phe Val Val Gly Gln Met Val Tyr Tyr Gln Cys Val Gln Gly Tyr Arg Ala Leu His 139
CAT TTC GTG GTG GGG CAG ATG GTT TAT TAT CAG TGC GTC CAG GGA TAC AGG GCT CTA CAC 480
Arg Gly Pro Ala Glu Ser Val Cys Lys Met Thr His Gly Lys Thr Arg Trp Thr Gln Pro 159
AGA GGT CCT GCT GAG AGC GTC TGC AAA ATG ACC CAC GGG AAG ACA AGG TGG ACC CAG CCC 540
Gln Leu Ile Cys Thr Gly Glu Met Glu Thr Ser Gln Phe Pro Gly Glu Glu Lys Pro Gln 179
CAG CTC ATA TGC ACA GGT GAA ATG GAG ACC AGT CAG TTT CCA GGT GAA GAG AAG CCT CAG 600
BglI
Ala Ser Pro Glu Gly Arg Pro Glu Ser Glu Thr Ser Cys Leu Val Thr Thr Thr Asp Phe 199
GCA AGC CCC GAA GGC CGT CCT GAG AGT GAG ACT TCC TGC CTC GTC ACA ACA ACA GAT TTT 660
Gln Ile Gln Thr Glu Met Ala Ala Thr Met Glu Thr Ser Ile Phe Thr Thr Glu Tyr Gln 219
CAA ATA CAG ACA GAA ATG GCT GCA ACC ATG GAG ACG TCC ATA TTT ACA ACA GAG TAC CAG 720
Val Ala Val Ala Gly Cys Val Phe Leu Leu Ile Ser Val Leu Leu Leu Ser Gly Leu Thr 239
GTA GCA GTG GCC GGC TGT GTT TTC CTG CTG ATC AGC GTC CTC CTC CTG AGT GGG CTC ACC 780
XbaI
Trp Gln Arg Arg Gln Arg Lys Ser Arg Arg Thr Ile End 251
TGG CAG CGC AGA CAG AGG AAG AGT AGA AGA ACA ATC TAG A--3' 862

FIG.2

FIG.3


A.
3'
5'
BamHI
XboI
PstI
SstI
PstI
pN1/N4-S
BamHI
XboI
SstI
PstI
pN1/N4-X
BglII
PstI
SV40
+E ori L
BamHI
PMLSV
BamHI
amp
PstI
B. 125I-Anti-Receptor Antibody
Specific CPM Bound (x10-3)
50
40
30
20
10
0
C. 125I-IL-2
8
6
4
2
0
pN1/ N4-S
pN1/ N4-X
Control
Transfected Cos-7 Cells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BamHI
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BglI
PstI
SstI
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XbaI
BglI
SstI
PstI
[PstI]
N1

FIG.1

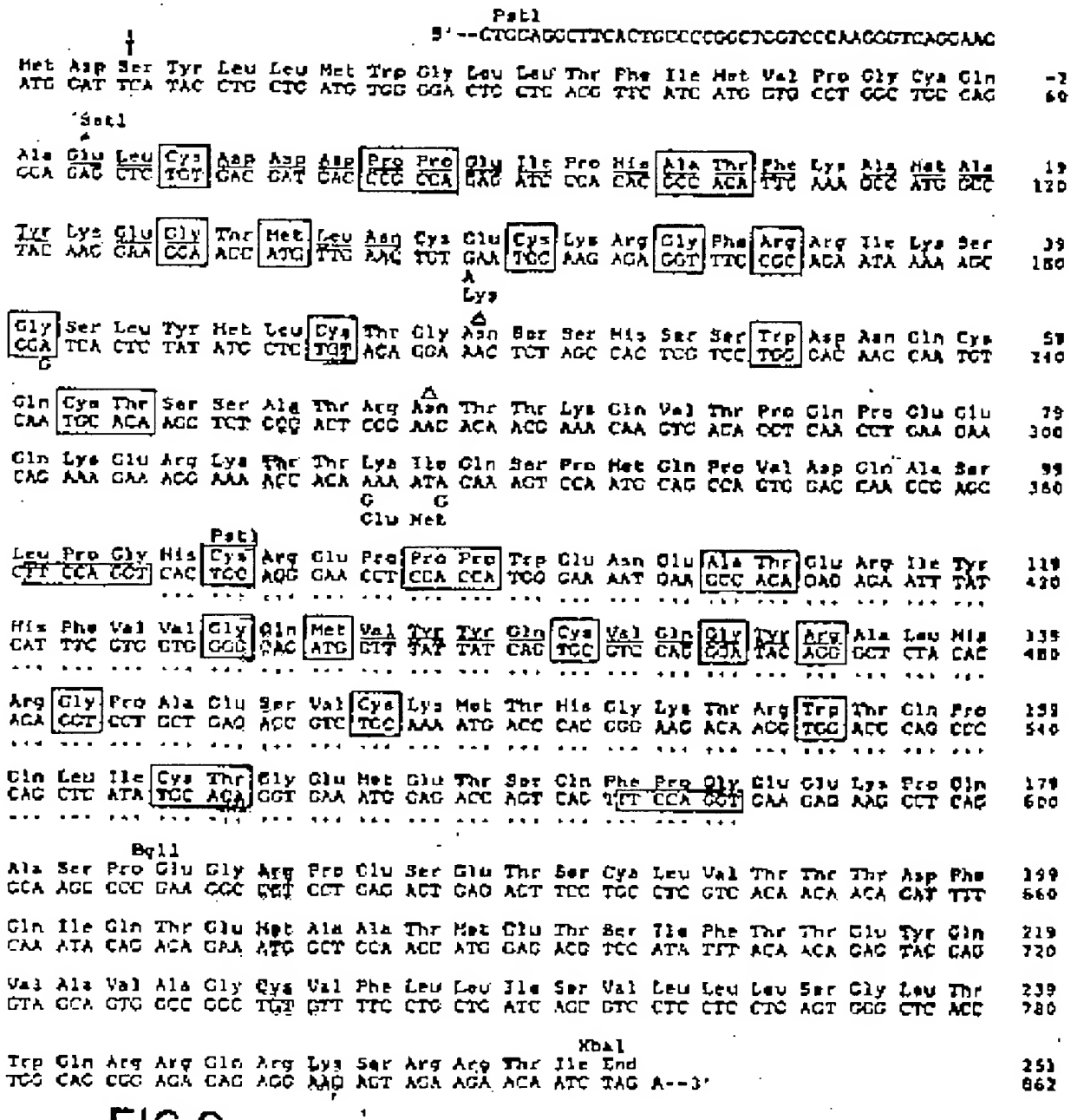
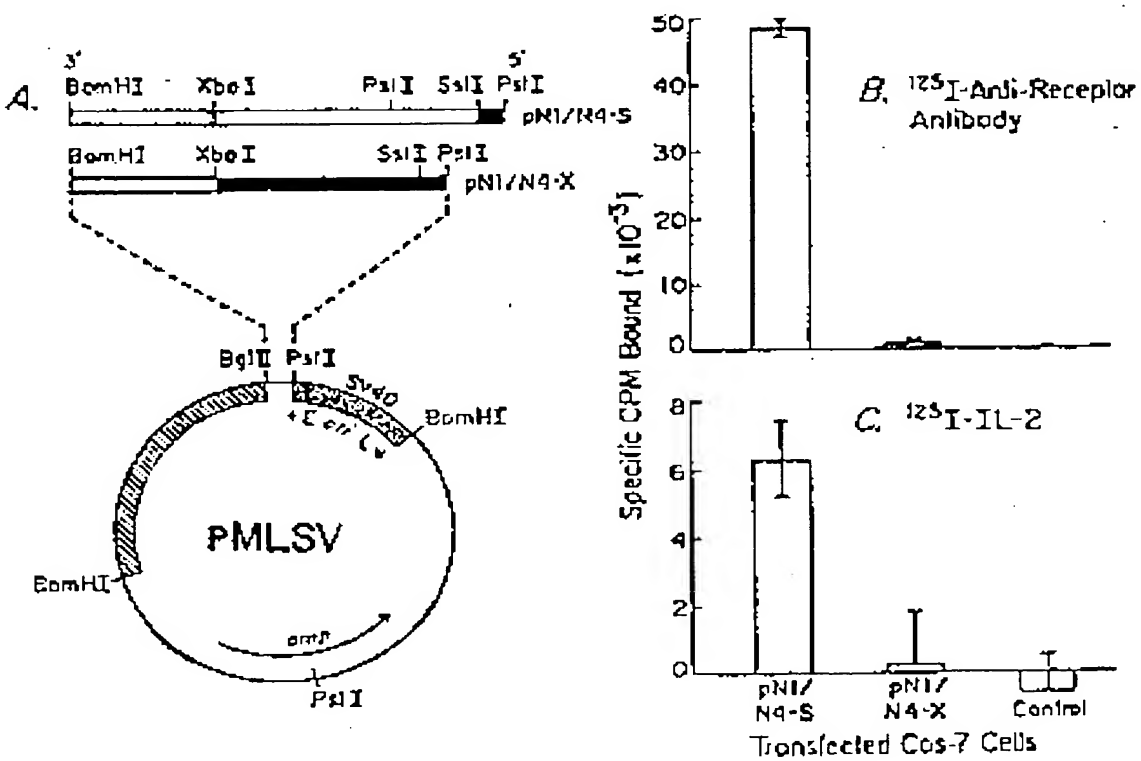

PstI
5'--CTGCAGGCTTCACTGCCCCGGCTCGTCCCAAGCGTCAGGAAC
Met Asp Ser Tyr Leu Leu Met Trp Gly Leu Leu Thr Phe Ile Met Val Pro Gly Cys Gln -2
ATG GAT TCA TAC CTG CTC ATG TGG GGA CTC CTC ACG TTC ATC ATG GTG CCT GGC TGC CAG 60
SstI
Ala Glu Leu Cys Asp Asp Asp Pro Pro Glu Ile Pro His Ala Thr Phe Lys Ala Met Ala 19
GCA GAG CTC TGT GAC GAT GAC CCG CCA GAG ATC CCA CAC GCC ACA TTC AAA GCC ATG GCC 120
Tyr Lys Glu Gly Thr Met Leu Asn Cys Glu Cys Lys Arg Gly Phe Arg Arg Ile Lys Ser 39
TAC AAG GAA GGA ACC ATG TTG AAC TGT GAA TGC AAG AGA GGT TTC CGC AGA ATA AAA AGC 180
A
Lys
Gly Ser Leu Tyr Met Leu Cys Thr Gly Asn Ser Ser His Ser Ser Trp Asp Asn Gln Cys 59
G GGA TCA CTC TAT ATG CTC TGT ACA GGA AAC TCT AGC CAC TCG TCC TGG GAC AAC CAA TGT 240
Gln Cys Thr Ser Ser Ala Thr Arg Asn Thr Thr Lys Gln Val Thr Pro Gln Pro Glu Glu 79
CAA TGC ACA AGC TCT GCG ACT CGC AAC ACA ACG AAA CAA GTC ACA CCT CAA CCT GAA GAA 300
Gln Lys Glu Arg Lys Thr Thr Lys Ile Gln Ser Pro Met Gln Pro Val Asp Gln Ala Ser 99
CAG AAA GAA ACG AAA ACC ACA AAA ATA CAA AGT CCA ATG CAG CCA GTG GAC CAA GCC AGC 360
G G
Glu Met
PstI
Leu Pro Gly His Cys Arg Glu Pro Pro Pro Trp Glu Asn Glu Ala Thr Glu Arg Ile Tyr 119
CTT CCA GGT CAC TGC AGG GAA CCT CCA CCA TGG GAA AAT GAA GCC ACA GAG AGA ATT TAT 420
His Phe Val Val Gly Gln Met Val Tyr Tyr Gln Cys Val Gln Gly Tyr Arg Ala Leu His 139
CAT TTC GTG GTG GGC CAG ATG GTT TAT TAT CAG TGC GTC CAG GGA TAC AGG GCT CTA CAC 480
Arg Gly Pro Ala Glu Ser Val Cys Lys Met Thr His Gly Lys Thr Arg Trp Thr Gln Pro 159
AGA GGT CCT GCT GAG AGC GTC TGC AAA ATG ACC CAC GGG AAG ACA AGG TGG ACC CAG CCC 540
Gln Leu Ile Cys Thr Gly Glu Met Glu Thr Ser Gln Phe Pro Gly Glu Glu Lys Pro Gln 179
CAG CTC ATA TGC ACA GGT GAA ATG GAG ACC AGT CAG TTT CCA GGT GAA GAG AAG CCT CAG 600
BglII
Ala Ser Pro Glu Gly Arg Pro Glu Ser Glu Thr Ser Cys Leu Val Thr Thr Thr Asp Phe 199
GCA AGC CCC GAA GGC CGT CCT GAG AGT GAG ACT TCC TGC CTC GTC ACA ACA ACA GAT TTT 660
Gln Ile Gln Thr Glu Met Ala Ala Thr Met Glu Thr Ser Ile Phe Thr Thr Glu Tyr Gln 219
CAA ATA CAG ACA GAA ATG GCT GCA ACC ATG GAG ACG TCC ATA TTT ACA ACA GAG TAC CAG 720
Val Ala Val Ala Gly Cys Val Phe Leu Leu Ile Ser Val Leu Leu Leu Ser Gly Leu Thr 239
GTA GCA GTG GCC GGC TGT GTT TTC CTG CTC ATC AGC GTC CTC CTC CTG AGT GGG CTC ACC 780
XbaI
Trp Gln Arg Arg Gln Arg Lys Ser Arg Arg Thr Ile End 251
TGG CAG CGC AGA CAG AGG AAG AGT AGA AGA ACA ATC TAG A--3' 862

FIG.2

FIG.3


A.
3'
BamHI
XbaI
PstI
SalI
PstI
5'
pN1/N4-S
BamHI
XbaI
SalI
PstI
pN1/N4-X
BglII
PstI
SV40
BamHI
PMLSV
BamHI
amp
PstI
B. 125I-Anti-Receptor Antibody
C. 125I-IL-2
Specific CPM Bound (x10-3)
50
40
30
20
10
0
8
6
4
2
0
pN1/ N4-S
pN1/ N4-X
Control
Transfected Cos-7 Cells

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/12, C07K 14/715, 14/47, C12N 15/70, 15/85, 1/20, 5/10, C07K 16/28, A61K 31/70

A1

(11) International Publication Number: WO 96/21732

(43) International Publication Date: 18 July 1996 (18.07.96)

(21) International Application Number: PCT/EP95/05123

(22) International Filing Date: 23 December 1995 (23.12.95)

(30) Priority Data:
95100201.3 9 January 1995 (09.01.95) EP
(34) Countries for which the regional or international application was filed: DE et al.

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(81) Designated States: CA, JP, MX, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: IL-2R-ASSOCIATED POLYPEPTIDE AND DNA MOLECULES CODING THEREFOR

(57) Abstract

The present invention relates to a polypeptide, p43, which is associated with the interleukin-2 receptor (IL-2R). It binds specifically to the β and γ subunits of IL-2R and is further capable of binding NAD^+. The invention is further related to nucleic acid molecules coding for p43 and to antibodies specifically binding p43.

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IL-2R-associated polypeptide and DNA molecules coding therefore

Field of the invention

The present invention relates to the polypeptide p43, to polypeptides which contain binding sites for at least two of NAD^+, interleukin 2 receptor (IL-2R) β-chain, or IL-2R γ-chain, to nucleic acid molecules containing the coding information for the aforementioned polypeptides, to antibodies specific for the aforementioned polypeptides, to antisense oligonucleotides, to pharmaceutical compositions containing the aforementioned polypeptides or nucleic acids, and to methods of producing the aforementioned polypeptides.

Background of the invention

Interleukin 2 (IL-2) plays a critical role in the regulation of proliferation and differentiation of hematopoietic cells (27, 29). IL-2 exerts its multiple biological activities through its binding to a specific cell surface receptor (IL-2R) (30), including protein tyrosine kinase (PTK) activation, and nuclear proto-oncogene expression which may be critical for cellular proliferation (16, 29). IL-2R contains at least three distinct subunits; the α-chain, the β-chain and the γ-chain (5, 9, 28). Among these subunits, both the IL-2R β- and γ-chains belong to a newly identified superfamily of cytokine receptors, characterized by four conserved cysteines and the sequence Trp-Ser-X-Trp-Ser (the "WS motif") in their extracellular domains (1, 2). Notably, none of the IL-2R subunits possesses any known catalytic activity such as PTK activity.

The expression of different combinations of the IL-2R subunits gives rise to various forms of IL-2R, each of which exhibiting different binding affinity to IL-2 (28). The "high-affinity" IL-2R (Kd; 10^{-11}M) consists of the heterotrimer α-, β- and γ-chains, the "intermediate-affinity" IL-2R (Kd; 10^{-9}) results from the heterodimer β- and γ-chains, whereas the "low-affinity" IL-2R (Kd; 10^{-8}) can be generated by expression of the α-chain alone. IL-2R β-chain possesses the largest cytoplasmic domain, consisting of 288 amino acids (a.a.) and was shown to play a critical role in IL-2 signal transduction (8). When the human IL-2R β-chain cDNA was introduced into murine IL-3-dependent pro-B cell line BAF-B03, which normally expresses the endogenous IL-2R α- and γ-chains, but not the β-chain, these cells were capable of proliferating in response to IL-2 (3, 8). Further expres-

sion studies with deletion mutant cDNAs of the IL-2R β-chain revealed that a restricted cytoplasmic region of the IL-2R β-chain, designated the "serine-rich" region (S-region), is indispensable for c-*myc* gene induction and for mitogenesis following IL-2 stimulation of the BAF-B03 cells (26). Another cytoplasmic region of the IL-2R β-chain, rich in acidic amino acids, designated the "acidic" region (A-region), is required in addition to the S-region for the *src*-family PTK activation and $p21^{ras}$ activation and for c-*fos*/c-*jun* gene induction following IL-2 stimulation of BAF-B03 cells (6, 7, 17, 24, 26). Several lines of evidence suggest that the IL-2R γ-chain may also be critical for IL-2-induced signal transduction (29). Moreover, IL-2R γ-chain is suggested to be a shared common component among the IL-2, IL-4 and IL-7 receptors and possibly other cytokine receptors (14, 15, 21, 23). Mutations of IL-2R γ-chain have been found in X-linked severe combined immunodeficiency patients who show defects in T-cell development (22), providing evidence for the critical role of IL-2R γ-chain in cytokine signaling. Furthermore, recent studies have indicated that the functional cooperation between the cytoplasmic domains of IL-2R β-chain and γ-chain is critical for IL-2 signaling (11, 19, 20).

Because of the importance of IL-2R-mediated processes for normal body functions and disease, there is a need of better understanding of these processes as well as the need of new tools for influencing them.

Disclosure of the invention

The present invention provides a new IL-2R-associated protein, p43, and nucleic acid molecules containing the coding information for p43. Preferably, the p43 polypeptide has the amino acid sequence of SEQ ID NO: 2 (cf. Fig. 1A):

Met Glu Phe Leu Lys Thr Cys Val Leu Arg Arg Asn Ala Cys Thr
Ala Val Cys Phe Trp Arg Ser Lys Val Val Gln Lys Pro Ser Val
Arg Arg Ile Ser Thr Thr Ser Pro Arg Ser Thr Val Met Pro Ala
Trp Val Ile Asp Lys Tyr Gly Lys Asn Glu Val Leu Arg Phe Thr
Gln Asn Met Met Met Pro Ile Ile His Tyr Pro Asn Glu Val Ile
Val Lys Val His Ala Ala Ser Val Asn Pro Ile Asp Val Asn Met
Arg Ser Gly Tyr Gly Ala Thr Ala Leu Asn Met Lys Arg Asp Pro
Leu His Val Lys Ile Lys Gly Glu Glu Phe Pro Leu Thr Leu Gly
Arg Asp Val Ser Gly Val Val Met Glu Cys Gly Leu Asp Val Lys
Tyr Phe Lys Pro Gly Asp Glu Val Trp Ala Ala Val Pro Pro Trp
Lys Gln Gly Thr Leu Ser Glu Phe Val Val Val Ser Gly Asn Glu

Val Ser His Lys Pro Lys Ser Leu Thr His Thr Gln Ala Ala Ser
Leu Pro Tyr Val Ala Leu Thr Ala Trp Ser Ala Ile Asn Lys Val
Gly Gly Leu Asn Asp Lys Asn Cys Thr Gly Lys Arg Val Leu Ile
Leu Gly Ala Ser Gly Gly Val Gly Thr Phe Ala Ile Gln Val Met
Lys Ala Trp Asp Ala His Val Thr Ala Val Cys Ser Gln Asp Ala
Ser Glu Leu Val Arg Lys Leu Gly Ala Asp Asp Val Ile Asp Tyr
Lys Ser Gly Ser Val Glu Glu Gln Leu Lys Ser Leu Lys Pro Phe
Asp Phe Ile Leu Asp Asn Val Gly Gly Ser Thr Glu Thr Trp Ala
Pro Asp Phe Leu Lys Lys Trp Ser Gly Ala Thr Tyr Val Thr Leu
Val Thr Pro Phe Leu Leu Asn Met Asp Arg Leu Gly Ile Ala Asp
Gly Met Leu Gln Thr Gly Val Thr Val Gly Ser Lys Ala Leu Lys
His Phe Trp Lys Gly Val His Tyr Arg Trp Ala Phe Phe Met Ala
Ser Gly Pro Cys Leu Asp Asp Ile Ala Glu Leu Val Asp Ala Gly
Lys Ile Arg Pro Val Ile Glu Gln Thr Phe Pro Phe Ser Lys Val
Pro Glu Ala Phe Leu Lys Val Glu Arg Gly His Ala Arg Gly Lys
Thr Val Ile Asn Val Val,

or SEQ ID NO: 4 (cf. Fig. 1B, mouse p43):

Met Gly Val Leu Lys Thr Cys Val Leu Arg Arg Ser Ala Cys Ala
Ala Ala Cys Phe Trp Arg Arg Thr Val Ile Pro Lys Pro Pro Phe
Arg Gly Ile Ser Thr Thr Ser Ala Arg Ser Thr Val Met Pro Ala
Trp Val Ile Asp Lys Tyr Gly Lys Asn Glu Val Leu Arg Phe Thr
Gln Asn Met Met Leu Pro Ile Ile His Tyr Pro Asn Glu Val Ile
Ile Lys Val His Ala Ala Ser Val Asn Pro Ile Asp Val Asn Met
Arg Ser Gly Tyr Gly Ala Thr Ala Leu Asn Met Lys Arg Asp Pro
Leu His Met Lys Thr Lys Gly Glu Glu Phe Pro Leu Thr Leu Gly
Arg Asp Val Ser Gly Val Val Met Glu Cys Gly Leu Asp Val Lys
Tyr Phe Gln Pro Gly Asp Glu Val Trp Ala Ala Val Pro Pro Trp
Lys Gln Gly Thr Leu Ser Glu Phe Val Val Val Ser Gly Asn Glu
Val Ser His Lys Pro Lys Ser Leu Thr His Thr Gln Ala Ala Ser
Leu Pro Tyr Val Ala Leu Thr Ala Trp Ser Ala Ile Asn Lys Val
Gly Gly Leu Ser Asp Arg Asn Cys Lys Gly Lys Arg Ala Leu Ile
Leu Gly Ala Ser Gly Gly Val Gly Thr Phe Ala Ile Gln Val Met
Lys Ala Trp Gly Ala His Val Thr Ala Val Cys Ser Lys Asp Ala
Ser Glu Leu Val Arg Lys Leu Gly Ala Asp Glu Val Ile Asp Tyr
Thr Leu Gly Ser Val Glu Glu Gln Leu Lys Ser Leu Lys Leu Cys
Ala Phe Ile Leu Asp Asn Val Gly Gly Ser Thr Glu Thr Trp Ala

Leu Asn Phe Leu Lys Lys Trp Ser Gly Ala Thr Tyr Val Thr Leu
Val Thr Pro Phe Leu Leu Asn Met Asp Arg Leu Gly Val Ala Asp
Gly Met Leu Gln Thr Gly Val Thr Val Gly Thr Lys Ala Met Lys
His Leu Trp Gln Gly Val His Tyr Arg Trp Ala Phe Phe Met Ala
Ser Gly Pro Tyr Leu Asp Glu Ile Ala Glu Leu Val Asp Ala Gly
Lys Ile Arg Pro Val Ile Glu Arg Thr Phe Pro Phe Ser Glu Val
Pro Glu Ala Phe Leu Lys Val Glu Arg Gly His Ala Arg Gly Lys
Thr Val Val Asn Val Val.

The invention is further related to polypeptides with p43-like activity or functional derivatives of p43, especially polypeptides which contain a binding site for at least NAD^+ and IL-2R β-chain, NAD^+ and IL-2R γ-chain, or IL-2R β-chain and IL-2R γ-chain. Functional derivatives may be variants, fragments, chemical derivatives, or fusion proteins of p43.

In a further aspect, the present invention is related to nucleic acid molecules containing the coding information for p43, polypeptides with p43-like activity, or functional derivatives. Preferably, a nucleic acid molecule according to the present invention is a nucleic acid molecule containing the nucleotide sequence of SEQ ID NO: 1 (cf. Fig. 1A):

AGAATGGACA GAATACTGAC TGGAACGTTA ATTCGAGCAT TTCATATGCG
AAGAGCGGAA TAACAGTTCC GTATTCTTCT TTCAGTTTCT CCATTAGATT
AGCTTCATTT TCGAAGGCTC CGTTTTGCAT GCTTAATTTT GAAACTAGCC
CGTGGTTTGG CAGAATTTGA CTGAATTCAG GGGTGAGAGT TTGATCCAGT
CCAAGTGTAT TTGAATTTGA GCACGCAGTT CAACCAGTGT TTACA
ATG GAA TTT CTG AAG ACT TGT GTA CTT AGA AGA AAT GCA TGC ACT
GCG GTT TGC TTC TGG AGA AGC AAA GTT GTC CAA AAG CCT TCA GTT
AGA AGG ATT AGT ACT ACC TCT CCA AGG AGC ACT GTC ATG CCT GCT
TGG GTG ATA GAT AAA TAT GGG AAG AAT GAA GTG CTT CGA TTC ACT
CAG AAC ATG ATG ATG CCT ATT ATA CAC TAT CCA AAT GAA GTC ATT
GTC AAA GTT CAC GCT GCC AGT GTA AAT CCT ATA GAC GTT AAT ATG
AGA AGT GGT TAT GGA GCT ACA GCT TTA AAT ATG AAG CGT GAT CCT
TTA CAC GTG AAA ATC AAA GGA GAA GAA TTT CCT CTG ACT CTG GGT
CGG GAT GTC TCT GGC GTG GTG ATG GAA TGT GGG CTT GAT GTG AAA
TAC TTC AAG CCT GGA GAT GAG GTC TGG GCT GCA GTT CCT CCT TGG
AAA CAA GGC ACT CTT TCA GAG TTT GTT GTA GTC AGT GGG AAT GAG
GTC TCT CAC AAA CCC AAA TCA CTC ACT CAT ACT CAA GCT GCC TCT

TTG CCA TAT GTG GCT CTC ACA GCC TGG TCT GCT ATA AAC AAA GTT
GGT GGC CTG AAT GAC AAG AAT TGC ACA GGA AAA CGT GTT CTA ATC
TTA GGC GCT TCA GGC GGA GTT GGT ACT TTT GCT ATA CAG GTA ATG
AAA GCA TGG GAT GCT CAT GTG ACA GCA GTT TGC TCC CAA GAT GCC
AGT GAA CTT GTA AGG AAG CTT GGT GCA GAC GAT GTA ATT GAT TAC
AAA TCT GGA AGT GTG GAA GAG CAG TTG AAA TCC TTA AAA CCA TTT
GAT TTT ATC CTT GAT AAT GTT GGC GGA TCC ACT GAA ACA TGG GCT
CCA GAT TTT CTC AAG AAA TGG TCA GGA GCC ACC TAT GTG ACT TTG
GTG ACT CCT TTC CTC CTG AAC ATG GAC CGA TTG GGC ATA GCA GAT
GGC ATG TTG CAG ACA GGA GTC ACT GTA GGT TCA AAG GCA TTA AAG
CAT TTC TGG AAA GGA GTC CAT TAT CGC TGG GCA TTT TTC ATG GCC
AGT GGC CCA TGT TTA GAT GAC ATT GCA GAA CTG GTG GAT GCG GGA
AAG ATC CGG CCA GTT ATT GAA CAA ACC TTT CCT TTT TCT AAA GTT
CCA GAA GCC TTC CTG AAG GTG GAA AGA GGA CAC GCA CGA GGA AAG
ACT GTA ATT AAT GTT GTT TAAATAAAAA TGCAGTTTAG TGATTAAAAA
AAAAAAAAAA AAAAAAAA,

or a degenerate variant of said nucleic acid molecule containing the nucleotide sequence of SEQ ID NO: 1, or a nucleic acid molecule capable of hybridizing to a nucleic acid molecule having SEQ ID NO: 1, or a nucleic acid molecule containing a part of the nucleotide sequence of any of the foregoing nucleic acid molecules, or a fragment of any one of the foregoing nucleic acid molecules. Preferably, such a nucleic acid molecule containing a part of SEQ ID NO: 1 contains the nucleotide sequence of SEQ ID NO: 9:

ATG GAA TTT CTG AAG ACT TGT GTA CTT AGA AGA AAT GCA TGC ACT
GCG GTT TGC TTC TGG AGA AGC AAA GTT GTC CAA AAG CCT TCA GTT
AGA AGG ATT AGT ACT ACC TCT CCA AGG AGC ACT GTC ATG CCT GCT
TGG GTG ATA GAT AAA TAT GGG AAG AAT GAA GTG CTT CGA TTC ACT
CAG AAC ATG ATG ATG CCT ATT ATA CAC TAT CCA AAT GAA GTC ATT
GTC AAA GTT CAC GCT GCC AGT GTA AAT CCT ATA GAC GTT AAT ATG
AGA AGT GGT TAT GGA GCT ACA GCT TTA AAT ATG AAG CGT GAT CCT
TTA CAC GTG AAA ATC AAA GGA GAA GAA TTT CCT CTG ACT CTG GGT
CGG GAT GTC TCT GGC GTG GTG ATG GAA TGT GGG CTT GAT GTG AAA
TAC TTC AAG CCT GGA GAT GAG GTC TGG GCT GCA GTT CCT CCT TGG
AAA CAA GGC ACT CTT TCA GAG TTT GTT GTA GTC AGT GGG AAT GAG
GTC TCT CAC AAA CCC AAA TCA CTC ACT CAT ACT CAA GCT GCC TCT
TTG CCA TAT GTG GCT CTC ACA GCC TGG TCT GCT ATA AAC AAA GTT
GGT GGC CTG AAT GAC AAG AAT TGC ACA GGA AAA CGT GTT CTA ATC

TTA GGC GCT TCA GGC GGA GTT GGT ACT TTT GCT ATA CAG GTA ATG
AAA GCA TGG GAT GCT CAT GTG ACA GCA GTT TGC TCC CAA GAT GCC
AGT GAA CTT GTA AGG AAG CTT GGT GCA GAC GAT GTA ATT GAT TAC
AAA TCT GGA AGT GTG GAA GAG CAG TTG AAA TCC TTA AAA CCA TTT
GAT TTT ATC CTT GAT AAT GTT GGC GGA TCC ACT GAA ACA TGG GCT
CCA GAT TTT CTC AAG AAA TGG TCA GGA GCC ACC TAT GTG ACT TTG
GTG ACT CCT TTC CTC CTG AAC ATG GAC CGA TTG GGC ATA GCA GAT
GGC ATG TTG CAG ACA GGA GTC ACT GTA GGT TCA AAG GCA TTA AAG
CAT TTC TGG AAA GGA GTC CAT TAT CGC TGG GCA TTT TTC ATG GCC
AGT GGC CCA TGT TTA GAT GAC ATT GCA GAA CTG GTG GAT GCG GGA
AAG ATC CGG CCA GTT ATT GAA CAA ACC TTT CCT TTT TCT AAA GTT
CCA GAA GCC TTC CTG AAG GTG GAA AGA GGA CAC GCA CGA GGA AAG
ACT GTA ATT AAT GTT GTT,

or a degenerate variant of SEQ ID NO: 9.

Preferably, a nucleic acid molecule according to the present invention is capable of hybridizing to a nucleic acid molecule having the nucleotide sequence of SEQ ID NO: 1 under conditions which select for a homology, or sequence identity, of more than 50 %, more preferably more than 70 %, more preferably more than 80 %, more preferably more than 90 %. Preferably, such nucleic acid molecules capable of hybridizing contain the coding information for polypeptides with p43-like biological and/or immunological activity, said polypeptides, more preferably, having at least one of the binding sites of p43 for NAD^+, IL-2R β-chain, or IL-2R γ-chain, more preferably at least two of said binding sites.

A further aspect of the present invention is a vector containing the nucleotide sequence of any one of the foregoing nucleic acids, especially when said nucleotide sequence is operationally linked to an expression control sequence as in expression vectors.

A further aspect of the present invention is a host cell carrying a vector as described, especially an expression vector. Such a host cell can be a procaryotic or eucaryotic cell. Preferably, such a host cell is a bacterial cell, a yeast cell, or a mammalian cell. More preferably, said host cell is an *E. coli* cell or a COS cell.

Accordingly, a still further aspect of the present invention is a method of production of p43, functional derivatives of p43, or polypeptides with p43-like activity, by recombinant expression. Such a method is characterized by cultivating a host cell as de-

scribed, said host cell carrying an expression vector containing the coding information for p43, a functional derivative of p43, or a polypeptide with p43-like biological activity, under conditions where said coding information is expressed by said host cell, and isolating the expressed polypeptide.

A further aspect of the present invention is an antibody molecule specific for p43, a functional derivative of p43, or a polypeptide with p43-like activity. Such an antibody molecule can be a polyclonal or monoclonal antibody, a complete immunoglobulin or a fragment thereof, especially a Fab' or $F(ab)_2$ fragment, a recombinant antibody or antibody fragment, for example a recombinant single-chain antibody (scFv), a chimeric, bispecific or humanised antibody.

Preferably, such an antibody molecule is specific for one of the following amino acid sequences:

SEQ ID NO:10: CKVVQKPSVRRISTTSPRST
SEQ ID NO:11: CYKSGSVEEQLKSLKPFDFI
SEQ ID NO:12: CGGSTETWAPDFLKKWSGAT,

SEQ ID NO: 11 being preferred.

A still further aspect of the present invention is an antisense oligonucleotide corresponding to a part of the nucleotide sequence of any nucleic acid molecule according to the present invention. One preferred embodiment of such an oligonucleotide has the sequence SEQ ID NO: 8: 5'-GTCTTCAAAACGCCCATCCT-3'.

A still further aspect of the present invention is a pharmaceutical composition containing p43, a functional derivative of p43, or a polypeptide with p43-like activity, or a nucleic acid containing the coding information for any one of the foregoing polypeptides, or an oligonucleotide corresponding to a part of the nucleotide sequence of said nucleic acid molecule. Such a pharmaceutical composition can be used for the treatment and diagnosis of IL-2-related disorders.

As used herein, a "polypeptide with p43-like activity" is a polypeptide which exhibits a biological activity which is essentially similar to p43. This means that is has one or more, preferably at least two of its structural or catalytic properties in common with p43, for example with respect to the binding properties of p43 to NAD^+, IL-2R β-chain, and/or IL-2R γ-chain. As used herein, a "functional derivative" of p43 is a compound which possesses

a biological activity (either functional or structural) that is substantially similar to a biological activity of p43. Examples of biological activities include the ability to bind to a natural ligand of p43, preferably to bind at least two of NAD^+, IL-2R β-chain, or IL-2R γ-chain. A molecule is said to be "substantially similar" to another molecule if both molecules have substantially similar structures or if both molecules possess a similar biological activity. The "functional derivatives" of p43 include fragments, variants, chemical derivatives or fusion proteins of p43. The term "fragment of p43" is meant to refer to any polypeptide subset of that molecule. The term "variant of p43" is meant to refer to a molecule substantially similar in structure to either the entire molecule, or to a fragment thereof, provided that the variant has at least one biological activity that is either similar to an activity of p43 or inhibitory to an activity of p43. A variant of p43 may differ from p43 by the substitution, deletion or addition of one or more amino acids, preferably 1 to 10 amino acids. Preferably, a variant has the ability to bind at least two of NAD^+, IL-2R β-chain, or IL-2R γ-chain. A "chemical derivative of p43" is a molecule which has been derived from p43 by a chemical reaction, for example iodination, acetylation, or linkage to a radioisotope or toxin. A "fusion protein of p43" is a polypeptide which has been generated by recombinant expression of all or a part of the p43 gene fused to all or part of another gene or nucleic acid containing in-frame coding information. A "degenerate variant" of a nucleic acid molecule is a second nucleic acid molecule which has a different nucleotide sequence as compared to the first nucleic acid molecule and codes for the same amino acid sequence as the first nucleic acid molecule, due to the degeneracy of the genetic code. A "fragment" of a nucleic acid molecule means a second nucleic acid molecule which has a nucleotide sequence representing a part of the nucleic acid sequence of the first nucleic acid molecule.

One way of carrying out the present invention is to isolate cDNAs whose protein products can interact with IL-2R γ-chain. To screen for human cDNA encoding proteins able to interact with IL-2R γ-chain, the two hybrid screening procedure described by (37) and (4) can be employed. In principle, DNA coding for IL-2R γ-chain (28), or a part of it, is fused to a DNA coding for the N-terminal domain of the *Saccharomyces cerevisiae* GAL4 protein, said N-terminal domain being capable of binding to specific DNA sequences (UAS_G). This construct can then be incorporated into an expression vector and transformed into a yeast strain which is deficient in GAL4. A cDNA collection which is to be screened can be incorporated into an expression vector, wherein the individual cDNA molecules are fused to DNA coding for the transcriptional activation domain of GAL4. The resulting constructs are transformed into the same yeast strain which has been pretransformed with the IL-2R γ-chain construct. In yeast cells which carry the cDNAs of interest, namely cDNAs coding for polypeptides able to bind to IL-2R γ-chain, those molecules bind to the IL-2R γ-chain polypeptides which are expressed in the same cell, thus bringing the two GAL4 do-

mains (the DNA binding domain fused to IL-2R γ-chain and the transcriptional activation domain fused to the cDNA of interest) together. As a result of this interaction, transcription of genes regulated by GAL4/UAS_G occurs. This can be employed for a suitable selection system, for example using the well-known β-galactosidase/galactose system. For example, LexA protein and IL-2R γ-chain fused gene can be constructed and transformed into appropriate yeast cells. The resultant transformant cell can be sequentially transformed with a pACT human cDNA library (4), and transformants can be subjected to the screening procedure. Transformants can be placed under selection, and surviving colonies can be screened for their ability to produce β-galactosidase. Positive clones consisting of a partial open reading frame fused to the GAL4 transcriptional activation domain can be identified. Using the cDNA insert of such a positive clone as probe, the overlapping cDNAs can be obtained. A full-length cDNA clone may be obtained or constructed from overlapping fragments by standard procedures. cDNAs obtained this way can then be used to screen other cDNA libraries, for example from other species like mouse, to identify related polypeptides. This can be performed by standard procedures as well.

Given the information of the present invention, especially the sequence information according to Fig. 1A and Fig. 1B, the polypeptides and nucleic acid molecules of the present invention can be produced by standard procedures. A nucleic acid molecule with the nucleotide sequence according to Fig. 1A, for example, can be produced by chemical synthesis. An alternative way would be to chemically synthesize an oligonucleotide or DNA fragment corresponding to a part of the nucleotide sequence as outlined in Fig. 1A and to screen an appropriate cDNA library or genomic library by hybridization. Detailed protocols how to design such an oligonucleotide or DNA fragment, how to generate a library, and how to screen such a library by hybridization with the oligonucleotide or DNA fragment can be found in standard laboratory manuals, for example in (32), especially in chapters 7, 8, 9, 11 and 12, the content of which shall be incorporated into this specification by reference. Therein, it is also taught how to adjust the appropriate hybridization conditions for a given probe, for example conditions which select for perfect matching (homology of 100%), or conditions which select for homologies of 50%, 70%, 80% or 90% (32, pages 11.45-11.57). As an example, using a human p43 cDNA as a probe, hybridization in 3 x SSC at 65°C could select mouse p43 cDNA which has a homology of about 90% on the amino acid level. Alternatively, a nucleic acid containing the coding information for p43, or a fragment thereof, can be generated from a cDNA library by polymerase chain reaction according to standard laboratory protocols (32, chapter 14).

With a nucleic acid coding for p43 or a functional derivative thereof in hands, especially the coding sequence according to Fig. 1A (starting with A at position 246 and ending

with T at position 1433 of the nucleotide sequence of Fig. 1A), the expert can produce the polypeptide by recombinant expression according to standard protocols either in procaryotic or eucaryotic host cells (see, for example, 32, especially chapters 16 and 17). For this purpose, the nucleic acid molecule containing the coding sequence of interest is incorporated into an expression vector where it is operationally linked to an expression control sequence. This expression vector is adapted to the special requirements of the host cell of choice. Expression may be regulatable. The expression vector is then introduced into the host cell of choice. Upon cultivation under appropriate conditions, the host cells synthesize the p43 polypeptide or functional derivative thereof. The expression system may permit secretion of the expressed polypeptide into the culture medium. The polypeptide can then be isolated from either the host cells or, when the expressed polypeptide is secreted into the medium, from the culture medium. Specific examples for the expression of p43 or functional derivatives thereof are described below.

Given the information of the present invention, especially the sequence information of Fig. 1A or Fig. 1B, the expert may construct functional derivatives of p43. This can be achieved by constructing a DNA molecule containing the coding information for a functional derivative, incorporating this DNA molecule into an expression vector, introducing this expression vector into a host cell and then expressing said DNA molecule coding for said functional derivative. For example, the expert can produce a fragment of a DNA molecule coding for p43, said DNA fragment containing only a part of the complete sequence, and express this fragment. For a functional analysis of the resulting polypeptide fragment, the expert can perform binding studies with the natural ligands of p43, NAD^+, IL-2R β-chain, or IL-2R γ-chain, either as described below, or with similar methods. Preferably, fragments of p43 retain at least one, more preferably at least two of the binding sites for NAD^+, IL-2R β-chain, or IL-2R γ-chain. For the production of variants, the expert can modifiy a DNA molecule containing all or part of the complete coding information for p43 by standard procedures, for example site-directed mutagenesis (32, especially chapter 15; 33, chapter 11, p 279-295), and express the thus modified DNA molecule as described. As an example, variants may be characterized by substitution, insertion or deletion of one, two, three, or more amino acids, as compared to p43 as described. After expression, the thus generated variant polypeptide can be tested whether it is functional as described. For the production of chemical derivatives of a given polypeptide, standard procedures may be used as well (see, for example, 33, chapter 9, p 225-245, and chapter 10, p 247-277). The generation of fusion proteins is described in the examples below.

Given the information of the present invention, especially the sequence information according to Fig. 1A or Fig. 1B, the expert can produce antibodies specific for p43, or

functional derivatives thereof, according to standard procedures (34, especially vol. 1, chapters 2, 3, 4). For use as an antigen, for example, a synthetic peptide representing a part of the amino acid sequences of SEQ ID NO: 2 or 4, or Fig. 1A or 1B, can be synthesized and used in an immunization protocol, optionally linked to a carrier. Another example for generating an antigen is the recombinant expression of p43 or a functional derivative thereof, optionally as a fusion protein, for example in *E. coli*. The expressed polypeptide or fusion protein - optionally purified - can then be used in an immunization scheme. Specific antibodies or - in the case of monoclonal antibodies - hybridomas which produce specific antibodies can then be selected by appropriate methods (35). Antibodies may either be monoclonal or polyclonal. Instead of an intact immunglobulin, fragments of immunglobulins may be used, for example Fab' or $F(ab)_2$ fragments. The production of recombinant antibodies or antibody fragments, chimeric antibodies, humanised antibodies, bispecific antibodies or single-chain antibodies for a given antigen are state of the art. Antibodies may be coupled to other agents, for example radioisotopes or toxins. Antibodies specific for p43, or functional derivatives thereof, are useful tools for studying the mechanism of IL-2-induced cellular events or can be used to block or impair the transmission of the IL-2-induced signal.

Antisense oligonucleotides can be chemically synthesized according to standard procedures.

P43, functional derivatives thereof, nucleic acids containing the coding information for p43 or functional derivatives thereof, antibodies specific for p43 or functional derivatives thereof, or antisense oligonucleotides corresponding to parts of the nucleotide sequence coding for p43 or functional derivatives thereof can be used as drugs for the manufacture of pharmaceutical compositions for therapy or diagnosis of IL-2-related disorders. The molecules of the present invention can be formulated according to known methods, wherein these materials are combined in admixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles, and their formulation, optionally with other human proteins included, e.g. human serum albumin, are described, for example, in (36). The pharmaceutical compositions may be administered to patients intravenously, intramuscularly, subcutaneously, enterally, or parenterally. Administration may be by continous infusion, or by single or multiple boluses. The dosage will vary depending upon such factors as the patients age, weight, height, sex, general medical condition, disease, etc. In general, it will be in the range of from about 1 pg/kg body weight to 10 mg/kg body weight of patient.

Recombinantly produced p43 or functional derivatives thereof may be also used to study the mechanism of IL-2-induced signal transduction.

Figure legends

Fig. 1. *Nucleotide sequence and complete predicted amino acid sequence of p43.* (A) The nucleotide and predicted amino acid sequence of human p43. The amino acid sequence is indicated in single-letter code. Conserved residues of predicted NAD^+ binding domain are underlined. Nucleotide numbers are on the left, and amino acid numbers on the right. (B) Alignment of human and mouse p43 amino acid sequences. The region of predicted NAD^+ binding domain shown is boxed.

Fig. 2. *Homology between p43 and Dehydrogenase members.* Sequences of Alcohol dehydrogenase, Lactate dehydrogenase and Glyceraldehydephosphate dehydrogenase are aligned to human or mouse p43; numbering is with respect to human p43. Identity with human or mouse p43 is indicated in open boxes.

Fig. 3. *p43 mRNA expression in human tissues.* Human tissue blot (Clontech, Palo Alto, Calfornia). Molecular sizes are indicated on the left (in kilobases).

Fig. 4. *Association of p43 with IL-2R in COS cells.* (A) Association of p43 with IL-2R γ-chain. Cell lysates were prepared from COS cells; COS cells transfected with CD4 γ plus LCK tag, CD4γ plus LCK-p43, CD4γM1 plus LCK tag or CD4γM1 plus LCK-p43. Aliquots of the respective cell lysates were immunoprecipitated with anti-CD4 antibody (OKT4) followed by anti Lck immunoblotting. (B) Association of p43 with IL-2R β-chain. Cell lysates were prepared from COS cells; COS cells transfected with LCK-p43 plus CD4γ , CD4γM1, CD4β, CD4βA or CD4βS.

Fig. 5. *Effect of p43 antisense oligodeoxynucleotide on the IL-2 induced DNA synthesis.* FWT-2 cells were analyzed for their ability to incorporate [^{3}H] thymidine in the presence of antisense oligodeoxynucleotide after IL-2 stimulation. The data are represented as the average of triplicate determinations.

Fig. 6. *NAD^+ binds to p43.* [^{32}P] NAD^+ was incubated with the filter, which was transferred with ovalbumin, the recombinant p43 and alcohol dehydrogenase proteins, in the binding buffer (see Materials and Methods). Then, the filter was washed and exposed.

Examples

Example 1: **Two hybrid screening and cDNA isolation**

Unless otherwise indicated, the protocol was adapted according to (4). For yeast two-hybrid screening, the open reading frame of human IL-2R γ-chain cytoplasmic region (28) was fused to the LexA DNA binding domain in the vector pBTM116 (4) by the following procedure; synthesized oligonucleotide primers (sense: 5'-ATTTCCCGGGGGAACG-GACGATGCCCCGAA-3', antisense: 5'-CTTCTGTCGACGGATTGGGGTTCAGGTTT-C-3'), which contained SmaI or SalI site, respectively, were used for PCR amplification of the cDNA encoding IL-2R γ-chain cytoplasmic region. The fragment was cut by SmaI and SalI and ligated with pBTM116. The resultant plasmid was cut by PstI and ligated to remove the C-terminal half region of γ-chain. The resultant plasmid was transformed into the CTYLD yeast strain as bait. This transformant strain was sequentially transformed with a B-cell derived pACT human cDNA library (4) which was kindly obtained from Dr. S. Elledge, and $1x10^6$ transformants were analyzed by the standard method as described (4). Rare surviving colonies were screened for their ability to produce β-galactosidase. One positive clone was identified. Sequence analysis of the clone termed clone 36 encodes a partial open reading frame of ~1.4 kb fused to the GAL4 transcriptional activation domain. Using this insert as probe, a human cDNA library generated from Jurkat cell mRNA was screened to obtain a full length cDNA for p43 coding sequence. For the isolation of the human full length cDNA, the λgt11 cDNA library was prepared with poly (A)+ RNA from TPA-induced Jurkat cells (a human T cell leukemia line), according to standard procedures (32). For screening, probe DNA was prepared by XhoI enzyme treatment (cutting) of p43 cDNA obtained in the two hybrid screening. Five overlapping clones were characterized and found to possess inserts from 0.5-2 kb. DNA sequencing was carried out using the dideoxynucleotide chain termination method. The clone representing the longest insert coding sequence was sequenced. The clone contained a 2.0 kb cDNA segment that overlapped about 1.4 kb with clone 36, extended about 0.6 kb further to the 5' end, and contained the AUG initiation codon. This revealed a potential open reading frame of 396 amino acid-polypeptide (Fig. 1A) with a predicted molecular size of 43 kd. We called this gene product, p43. A computer-assisted sequence search with the GenBank database revealed that the sequence of p43 bears no significant homology to any known protein, except a partial similarity in a NAD^+ binding domain.

In addition, to obtain the mouse p43 cDNA, a cDNA library generated from mouse spleen cells mRNA was utilized and screened with human p43 cDNA fragment as probe. For the isolation of mouse p43 cDNA clone, the hybridization was performed using the

EcoRI-cleaved insert from the full length human p43 cDNA clone as a probe and using a mouse cDNA library as described (38), and the filters were washed in 3 x SSC at 65°C (SSC buffer was prepared according to ref. 32, p. B.12). Screening of the cDNA library from mouse spleen with human p43 cDNA yielded a clone of highly related sequence. The predicted human and mouse p43 amino acid sequences are highly related, showing ~90 % identity at the protein level (Fig. 1B). The amino acid sequence of NAD^+ binding domain is conserved in both human and mouse p43 (Fig. 1B and 2).

Expression of p43 mRNA detected by Northern blot analysis was ubiquitous to all human tissues tested. The p43 mRNA is approximately 2.0 kb in length and was most abundant in skeletal muscle (Fig. 3).

Example 2: **Association of p43 with IL-2R γ-chain in mammalian cells**

As mentioned above, we isolated an IL-2R γ-chain associated molecule, p43, using yeast two-hybrid system. However we did not know whether p43 can associate with IL-2R γ-chain in mammalian cells. To confirm the binding of p43 and IL-2R γ-chain in mammalian cells, we constructed two chimeric proteins linking p43 to the specific antibody recognized N-terminal region of $p56^{lck}$ (LCK-p43) and IL-2R γ-chain to the extracellular domain of CD4 (CD4γ). The expression plasmids, CD4γ and LCK-p43, were transiently co-transfected into monkey COS7 cells and then the intermolecular association was analyzed by immunoprecipitation with OKT4 and following western blotting analysis with anti-Lck antiserum (for details, see Example 6; see also ref. (7)). LCK-p43 and LCK tag were expressed in the transfected COS cells as assessed by anti-Lck antiserum immunoblotting of whole cell lysates (data not shown). Fig. 4 shows that IL-2R γ-chain bound to LCK-p43, but not to control LCK tag or the truncated IL-2R γ-chain, which contains only the transmembrane region, indicating a direct association of the two proteins in mammalian cells.

Example 3: **IL-2R β-chain also associates with p43**

Because IL-2R γ-chain and β-chain are associated and share important functions in IL-2 signal transduction, we determined whether IL-2R β-chain also associates with p43. To confirm the association of these molecules, we further constructed the chimera genes fusing IL-2R β-chain, or mutant β-chain, to CD4 (CD4-IL-2Rβ, CD4-βS, CD4-βA). These plasmids and Lck-p43 were cotransfected into monkey COS7 cells and the intermolecular association measured (cf. Examples 2, 6). Fig. 4B shows that p43 can associate with not

only IL-2R γ-chain but also IL-2R β-chain. Interestingly, the p43 was tightly associated with IL-2R β-chain through the S-region which is the critical region for IL-2-mediated signal transduction.

Example 4: **Synthesis of oligodeoxynucleotides and measurement of [^{3}H] thymidine incorporation**

S-oligodeoxynucleotides were synthesized on an automated DNA synthesizer (Applied Biosystems). The sequence of the sense and antisense oligodeoxynucleotides are: 5'-CAGGATGGGCGTTTTGAAGA-3' and 5'-GTCTTCAAAACGCCCATCCT-3', respectively. The FWT-2 cell, which is BAF-B03-derived cell line expressing wild-type human IL-2R β- and γ-chains, was used in this experiment. After continuously growing cells were washed with PBS, the cells were distributed into 96-well plates at an initial concentration of $1x10^4$ per well. An oligomer (5 μM or 10 μM) was added with or without IL-2 (2 nM). After 20 hrs incubation, the cells were pulse-labeled with 1 μCi of [^{3}H] thymidine (20 μCi/mmol) (NEN Research Products) 4 hrs prior to harvest.

The effect of p43 sense and antisense oligomers in [^{3}H] thymidine incorporation after IL-2 stimulation as a parameter of growth was evaluated as shown in Fig. 5 . The experiments have been repeated at least three times. It is evident that the p43 antisense oligodeoxynucleotide partially inhibits the [^{3}H] thymidine incorporation (~30%). On the other hand, no effect was observed using sense oligodeoxynucleotides. These results suggest that p43 molecule alters the IL-2 signal partially, but does not inhibit the full scale signal. It remains to evaluate the presence of redundant associated molecules, which can compensate for the absence of p43 in the presence of antisense oligonucleotides.

Example 5: **NAD^+ binding assay**

As mentioned earlier, p43 has partial homology to NAD^+ binding proteins, such as alcohol dehydrogenase. To confirm whether p43 can bind NAD^+, we performed NAD^+ binding assay. We first tested the binding of NAD^+ to recombinant p43 produced in bacteria. The complete open reading frame of p43 was fused to 6xHis tag sequences, and the resulting chimeric protein was purified from overexpressing bacterial strains by affinity chromatography on Ni-column. For the recombinant *E. coli* expressing p43, the p43 chimeric protein fused to the 6xHis affinity tag was constructed using vector, 6HisT-pET11d, which was kindly obtained from Mr. Hashimoto (Rockefeller University). Plasmid was trans-

formed into *E. coli* strain BL21/pLysS, and recombinant p43 was purified using Ni-column (Invitrogen). Purified p43 and control proteins (ovalbumin and alcohol dehydrogenase) were applied to SDS-PAGE (10-20 % gradient gel), and electrophoretically transferred onto PVDF membrane filters. After soaking in the binding buffer [50 mM Tris-HCl pH7.5, 1 mM EDTA, 5 mM $MgCl_2$, 0.3 % (v/v) Tween 20] at room temperature for 1 hr, the membrane filters were incubated with [^{32}P] NAD in the binding buffer at 20°C for 18 hrs. The filters were then washed with the binding buffer three times and exposed to X-ray film. As shown in Fig. 6, we detected the NAD^+ binding ability of p43. On the other hand, its binding ability could be completely abolished in the presence of deleted excess cold NAD.

Example 6: **Immunoprecipitation and immunoblotting analysis**

For immunoprecipitation and immunoblotting of IL-2R and p43, chimeric genes were constructed as follows: the CD4β, CD4βA, CD4βS, CD4γ and CD4γM1 chimeric receptor expression plasmids, which bear CD4 extracellular and transmembrane domains and the cytoplasmic domains of IL-2R β-chain, IL-2R β-chain lacking the internal "S-region" and the "A-region", IL-2R γ-chain, and the membrane proximal 7 amino acids of the cytoplasmic domain of IL-2R γ-chain, respectively, were constructed as described previously (18). Briefly, the CD4ß and CD4γ chimeric receptors are comprised of human CD4 extracellular/transmembrane domains, fused in frame with the cytoplasmic domains of IL-2Rß and IL-2Rγ chains respectively, using the two pairs of synthesized oligonucleotides. The CD4ß and CD4γ cDNAs were inserted into the EcoRI/XbaI cleaved pEF vector (25) (pEF-CD4ß and pEF-CD4γ) respectively. To construct the expression vectors (pEF-CD4ßA and CD4ßS) for the chimeric molecules, CD4ßA and CD4ßS, the pdKCRA and pdKCRS vectors (8), respectively, were digested with NcoI and BamHI. After digestion, the respective NcoI-BamHI fragments (~0.9Kb) were inserted into the NcoI/BamHI-cleaved pEF-CD4ß vector. The LCK-p43 chimeric molecule is comprised of the $p56^{lck}$ N-terminal region (~100 amino acids), mutated in the myristilation site from Gly to Ala, fused in frame with p43 using the PCR fragment of LCK. The LCK-p43 cDNA was inserted into pEF vector (25). The constructs were confirmed by restriction enzyme digestion and DNA sequencing.

The experiments for transient cDNA expression studies in COS cells were performed as described previously (7). The immunoprecipitation using anti-CD4 antibody (OKT4) and immunoblotting analysis using anti $p56^{lck}$ antiserum were also performed as described previously (18).

Example 7: **Production of antibodies**

Polyclonal antibodies have been raised against the following synthetic peptides corresponding to different sequence motifs of p43:

SEQ ID NO: 10:	CKVVQKPSVRRISTTSPRST (a.a. 23-41)
SEQ ID NO: 11:	CYKSGSVEEQLKSLKPFDFI (a.a. 255-273)
SEQ ID NO: 12:	CGGSTETWAPDFLKKWSGAT (a.a. 278-296)

The protocol of antigenic conjugate preparation, immunization and antibody titer determination is as follows:

The protocol for the conjugation of sulfhydryl-containing peptides to the carrier protein KLH was utilized. In brief, 1 mg of peptide and keyhole limpet hemocyamine (KLH, cf. 34, vol. I, p. 26) in 500 µl of PBS were mixed with 500 µl of complete Freund's adjuvant using Luer-lock connected syringes. After testing the proper mixing of the conjugate components, rabbits were injected subcutaneously in the back of the neck. The animals were boosted with 500 µg of antigen (conjugate mixed with incomplete Freund's adjuvant), at intervals of two weeks for a period of three months.

The antiserum was periodically tested using an enzyme-linked immunosorbant assay (ELISA), where the peptide KLH conjugate at 1 µg/µl was coated into ninety-six well microplates in coating buffer (0.1 M $NaHCO_3$ pH 9.0). After washing the microplate with rinse buffer (PBS, 0.1% Tween 20), different dilutions of the immune sera or control sera were added in a volume of 50-100 µl to the wells and incubated at room temperature for two hours.

The plates were washed with rinse buffer and 50 µl of goat anti-rabbit IgG conjugated with alkaline phospatase in PBS and 1% BSA and 0.1% Tween 20 was added to the wells and incubated for two hours at room temperature. The microplate was washed with rinse buffer and 100 µl of substrate, p-phenyl phosphate disodium in substrate buffer was added to the wells. The microplate was incubated at room temperature for 1-2 hours and the optical density (OD) at 405 nm measured (ref. 620 nm).

The three different anti-peptide antibodies could recognize the peptide conjugate at a dilution of $1/10^4$. The antibody against peptide SEQ ID NO. 11 could also recognize the *E. coli* expressed recombinant human p43.

References

1. **Bazan, J. F.** 1990. Structural design and molecular evolution of a cytokine receptor superfamily. Proc. Natl. Acad. Sci. USA **87**:6934-6938.

2. **Cosman, D., S. D. Lyman, R. L. Idzerda, M. P. Beckmann, L. S. Park, R. G. Goodwin, and C. J. March.** 1990. A new cytokine receptor superfamily. Trends Biochem. Sci. **15**:265-269.

3. **Doi, T., M. Hatakeyama, S. Minamoto, T. Kono, H. Mori, and T. Taniguchi.** 1989. Human interleukin 2 (IL-2) receptor β chain allows transduction of IL-2-induced proliferation signal(s) in a murine cell line. Eur. J. Immunol. **19**:2375-2378.

4. **Durfee, T., K. Becherer, P.-L. Chen, S.-H. Yeh, Y. Yang, A. E. Kilburn, W.-H. Lee, and S. J. Elledge.** 1993. The retinoblastoma protein associates with the protein phophatase type 1 catalytic subunit. Genes Dev. 7:555-569.

5. **Greene, W. C. and W. J. Leonard.** 1986. The human interleukin-2 receptor. Annu. Rev. Immunol. **4**:69-96.

6. **Hatakeyama, M., A. Kawahara, H. Mori, H. Shibuya, and T. Taniguchi.** 1992. *c-fos* gene induction by IL-2: identification of the critical cytoplasmic regions within the IL-2 receptor β chain. Proc. Natl. Acad. Sci. USA **89**:2022-2026.

7. **Hatakeyama, M., T. Kono, N. Kobayashi, A. Kawahara, S. D. Leven, R. M. Parlmutter, and T. Taniguchi.** 1991. Interaction of the IL-2 receptor with the *src*-family kinase $p56^{lck}$: Identification of novel intermolecular association. Science **252**:1523-1528.

8. **Hatakeyama, M., H. Mori, T. Doi, and T. Taniguchi.** 1989. A restricted cytoplasmic region of IL-2 receptor β chain is essential for growth signal transduction but not for ligand binding and internalization. Cell **59**:837-845.

9. **Hatakeyama, M., M. Tsudo, S. Minamoto, T. Kono, T. Doi, T. Miyata, M. Miyasaka, and T. Taniguchi.** 1989. Interleukin-2 receptor β chain gene: Generation of three receptor forms by cloned human α and β chain cDNAs. Science **244**:551-556.

10. **Johnston, J. A., M. Kawamura, R. A. Kirken, Y.-Q. Chen, T. B. Ortaldo, D. W. McVicar, and J. J. O'Shea.** 1994. Phosphorylation and activation of the Jak-3 Janus kinase in response to interleukin-2. Nature **370**:151-153.

11. **Kawahara, A., Y. Minami, and T. Taniguchi.** 1994. Evidence for a critical role for the cytoplasmic region of the interleukin 2 (IL-2) receptor γ chain in IL-2, IL-4 and IL-7 signalling. Mol. Cell. Biol. **14**:5433-5440.

12. **Kim, H., E. L. Jacobson, and M. K. Jacobson.** 1993. Synthesis and degradation of cyclic ADP-ribose by NAD glycohydrolases. Science **261**:1330-1333.

13. **Kobayashi, N., T. Kono, M. Hatakeyama, Y. Minami, T. Miyazaki, R. M. Perlmutter, and T. Taniguchi.** 1993. Functional coupling of the *src*-family protein tyrosine kinases p59*fyn* and p53/56*lyn* with the interleukin 2 receptor: Implications for redundancy and pleiotropism in cytokine signal transduction. Proc. Natl. Acad. Sci. USA **90**:4201-4205.

14. **Kondo, M., T. Takeshita, M. Higuchi, M. Nakamura, T. Sudo, S. Nishikawa, and K. Sugamura.** 1994. Functional participation of the IL-2 receptor γ chain in IL-7 receptor complexes. Science **263**:1453-1454.

15. **Kondo, M., T. Takeshita, N. Ishii, M. Nakamura, S. Watanabe, K. Arai, and K. Sugamura.** 1993. Sharing of the interleukin-2 (IL-2) receptor γ chain between receptors for IL-2 and IL-4. Science **262**:1874-1877.

16. **Minami, Y., T. Kono, T. Miyazaki, and T. Taniguchi.** 1993. The IL-2 receptor complex: Its structure, function, and target genes. Annu. Rev. Immunol. **11**:245-267.

17. **Minami, Y., T. Kono, K. Yamada, N. Kobayashi, A. Kawahara, R. M. Perlmutter, and T. Taniguchi.** 1993. Association of p56^{lck} with IL-2 receptor β chain is critical for the IL-2-induced activation of p56^{lck}. EMBO J. **12**:759-768.

18. **Minami, Y., Y. Nakagawa, A. Kawahara, T. Miayazaki, K. Sada, H. Yamamura, and T. Taniguchi.** 1995. Protein tyrosine kinase Syk is associated with and activated by the IL-2 receptor; possible link with the c-*myc* induction pathway. Immunity **2**(1):89-100.

19. **Nakamura, Y., S. M. Russell, S. A. Mess, M. Friedmann, M. Erdos, C. Francois, Y. Jacques, S. Adelstein, and W. J. Leonard.** 1994. Heterodimerization of the IL-2 receptor β- and γ-chain cytoplasmic domains is required for signalling. Nature **369**:330-333.

20. **Nelson, B. H., J. D. Lord, and P. D. Greenberg.** 1994. Cytoplasmic domains of the interleukin-2 receptor β and γ chains mediate the signal for T-cell proliferation. Nature **369**:333-336.

21. **Noguchi, M., Y. Nakamura, S. M. Russell, S. F. Ziegler, M. Tsang, X. Cao, and W. J. Leonard.** 1993. Interleukin-2 receptor γ chain: a functional component of the interleukin-7 receptor. Science **262**:1877-1880.

22. **Noguchi, M., H. Yi, H. M. Rosenblatt, A. H. Filipovich, S. Adelstein, A. S. Modi, O. W. McBride, and W. J. Leonard.** 1993. Interleukin-2 receptor γ chain mutation results in X-linked severe combined Immunodeficiency in human. Cell **73**:147-157.

23. **Russell, S. M., A. D. Keegan, N. Harada, Y. Nakamura, M. Noguchi, P. Leland, M. C. Friedmann, A. Miyajima, R. K. Puri, W. E. Poul, and W. J. Leonard.** 1993. Interleukin-2 receptor γ chain: a functional component of the interleukin-4 receptor. Science **262**:1880-1883.

24. **Satoh, T., Y. Minami, T. Kono, K. Yamada, A. Kawahara, T. Taniguchi, and Y. Kaziro.** 1992. Interleukin 2-induced activation of *ras* requires two domains of Interleukin 2 receptor β subunit, the essential region for growth stimulation and *lck*-binding domain. J. Biol. Chem. **267**:25423-25427.

25. **Shibuya, H., K. Irie, J. Ninomiya-Tsuji, M. Goebl, T. Taniguchi, and K. Matsumoto.** 1992. New human gene encoding a positive modulator of HIV Tat-mediated transactivation. Nature **357**:700-702.

26. **Shibuya, H., M. Yoneyama, J. Ninomiya-Tsuji, K. Matsumoto, and T. Taniguchi.** 1992. IL-2 and EGF receptors stimulate the hematopoietic cell cycle via different signaling pathways: Demonstration of a novel role for *c-myc*. Cell **70**:57-67.

27. **Smith, K. A.** 1988. Interleukin 2: inception, impact and implications. Science **240**:1169-1176.

28. **Takeshita, T., H. Asao, K. Ohtani, N. Ishii, S. Kumaki, N. Tanaka, H. Munakata, M. Nakamura, and K. Sugamura.** 1992. Cloning of the γ chain of the human IL-2 receptor. Science **257**:379-382.

29. **Taniguchi, T. and Y. Minami.** 1993. The IL-2/IL-2 receptor system: A current overview. Cell **73**:5-8.

30. **Waldmann, T. A.** 1989. The multi-subunit interleukin-2 receptor. Annu. Rev. Biochem. **58**:875-911.

31. **Witthuhn, B. A., O. Silvennoinen, O. Miura, K. S. Lai, C. Cwik, E. T. Liu, and J. N. Ihle.** 1994. Involvement of the Jak3 Janus kinase in signalling by interleukin 2 and 4 in lymphoid and myeloid cells. Nature **370**:153-157.

32. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning. A laboratory manual. Second edition. Cold Spring Harbour Laboratory Press.

33. **Creighton, T. E. (ed.).** 1989. Protein function - a practical approach. IRL press, Oxford.

34. **Catty, D. (ed.).** 1989. Antibodies - a practical approach. Vols. 1 and 2. IRL press, Oxford.

35. **Wunderlich, D., A. Lee, R. P. Fracasso, D. V. Mierz, R. M. Bayney, T. V. Ramabhadran.** 1992. Use of recombinant fusion proteins for generation and rapid characterization of monoclonal antibodies. J. Immunol. Methods **147**:1-11.

36. **Osol, A. (ed.).** 1980. Remington's Pharmaceutical Sciences (16th ed.). Mack, Easton, PA.

37. **Fields, S., O. Song.** 1989. A novel genetic system to detect protein-protein interactions. Nature **340**: 245-246.

38. **Kono T. et al.** 1989. Proc. Natl. Acad. Sci. U.S.A. **87**: 1806-1810.

Claims

1. A polypeptide with p43-like biological activity.

2. A polypeptide containing a NAD^+ binding domain and a binding site for the IL-2R β-chain.

3. The polypeptide of claim 2 which contains an additional binding site for IL-2R γ -chain.

4. A polypeptide containing a NAD^+ binding domain and a binding site for the IL-2R γ-chain.

5. A polypeptide containing a binding site for IL-2R β-chain and a binding site for IL-2R γ-chain.

6. The polypeptide of claims 3 or 5, wherein said polypeptide binds stronger to IL-2R β-chain than to IL-2R γ-chain.

7. The polypeptide of any one of claims 1 to 6 wherein said polypeptide has the amino acid sequence of SEQ ID NO: 2:

Met Glu Phe Leu Lys Thr Cys Val Leu Arg Arg Asn Ala Cys Thr
Ala Val Cys Phe Trp Arg Ser Lys Val Val Gln Lys Pro Ser Val
Arg Arg Ile Ser Thr Thr Ser Pro Arg Ser Thr Val Met Pro Ala
Trp Val Ile Asp Lys Tyr Gly Lys Asn Glu Val Leu Arg Phe Thr
Gln Asn Met Met Met Pro Ile Ile His Tyr Pro Asn Glu Val Ile
Val Lys Val His Ala Ala Ser Val Asn Pro Ile Asp Val Asn Met
Arg Ser Gly Tyr Gly Ala Thr Ala Leu Asn Met Lys Arg Asp Pro
Leu His Val Lys Ile Lys Gly Glu Glu Phe Pro Leu Thr Leu Gly
Arg Asp Val Ser Gly Val Val Met Glu Cys Gly Leu Asp Val Lys
Tyr Phe Lys Pro Gly Asp Glu Val Trp Ala Ala Val Pro Pro Trp
Lys Gln Gly Thr Leu Ser Glu Phe Val Val Val Ser Gly Asn Glu
Val Ser His Lys Pro Lys Ser Leu Thr His Thr Gln Ala Ala Ser
Leu Pro Tyr Val Ala Leu Thr Ala Trp Ser Ala Ile Asn Lys Val
Gly Gly Leu Asn Asp Lys Asn Cys Thr Gly Lys Arg Val Leu Ile
Leu Gly Ala Ser Gly Gly Val Gly Thr Phe Ala Ile Gln Val Met
Lys Ala Trp Asp Ala His Val Thr Ala Val Cys Ser Gln Asp Ala

Ser Glu Leu Val Arg Lys Leu Gly Ala Asp Asp Val Ile Asp Tyr
Lys Ser Gly Ser Val Glu Glu Gln Leu Lys Ser Leu Lys Pro Phe
Asp Phe Ile Leu Asp Asn Val Gly Gly Ser Thr Glu Thr Trp Ala
Pro Asp Phe Leu Lys Lys Trp Ser Gly Ala Thr Tyr Val Thr Leu
Val Thr Pro Phe Leu Leu Asn Met Asp Arg Leu Gly Ile Ala Asp
Gly Met Leu Gln Thr Gly Val Thr Val Gly Ser Lys Ala Leu Lys
His Phe Trp Lys Gly Val His Tyr Arg Trp Ala Phe Phe Met Ala
Ser Gly Pro Cys Leu Asp Asp Ile Ala Glu Leu Val Asp Ala Gly
Lys Ile Arg Pro Val Ile Glu Gln Thr Phe Pro Phe Ser Lys Val
Pro Glu Ala Phe Leu Lys Val Glu Arg Gly His Ala Arg Gly Lys
Thr Val Ile Asn Val Val,

or SEQ ID NO: 4:

Met Gly Val Leu Lys Thr Cys Val Leu Arg Arg Ser Ala Cys Ala
Ala Ala Cys Phe Trp Arg Arg Thr Val Ile Pro Lys Pro Pro Phe
Arg Gly Ile Ser Thr Thr Ser Ala Arg Ser Thr Val Met Pro Ala
Trp Val Ile Asp Lys Tyr Gly Lys Asn Glu Val Leu Arg Phe Thr
Gln Asn Met Met Leu Pro Ile Ile His Tyr Pro Asn Glu Val Ile
Ile Lys Val His Ala Ala Ser Val Asn Pro Ile Asp Val Asn Met
Arg Ser Gly Tyr Gly Ala Thr Ala Leu Asn Met Lys Arg Asp Pro
Leu His Met Lys Thr Lys Gly Glu Glu Phe Pro Leu Thr Leu Gly
Arg Asp Val Ser Gly Val Val Met Glu Cys Gly Leu Asp Val Lys
Tyr Phe Gln Pro Gly Asp Glu Val Trp Ala Ala Val Pro Pro Trp
Lys Gln Gly Thr Leu Ser Glu Phe Val Val Val Ser Gly Asn Glu
Val Ser His Lys Pro Lys Ser Leu Thr His Thr Gln Ala Ala Ser
Leu Pro Tyr Val Ala Leu Thr Ala Trp Ser Ala Ile Asn Lys Val
Gly Gly Leu Ser Asp Arg Asn Cys Lys Gly Lys Arg Ala Leu Ile
Leu Gly Ala Ser Gly Gly Val Gly Thr Phe Ala Ile Gln Val Met
Lys Ala Trp Gly Ala His Val Thr Ala Val Cys Ser Lys Asp Ala
Ser Glu Leu Val Arg Lys Leu Gly Ala Asp Glu Val Ile Asp Tyr
Thr Leu Gly Ser Val Glu Glu Gln Leu Lys Ser Leu Lys Leu Cys
Ala Phe Ile Leu Asp Asn Val Gly Gly Ser Thr Glu Thr Trp Ala
Leu Asn Phe Leu Lys Lys Trp Ser Gly Ala Thr Tyr Val Thr Leu
Val Thr Pro Phe Leu Leu Asn Met Asp Arg Leu Gly Val Ala Asp
Gly Met Leu Gln Thr Gly Val Thr Val Gly Thr Lys Ala Met Lys
His Leu Trp Gln Gly Val His Tyr Arg Trp Ala Phe Phe Met Ala
Ser Gly Pro Tyr Leu Asp Glu Ile Ala Glu Leu Val Asp Ala Gly

Lys Ile Arg Pro Val Ile Glu Arg Thr Phe Pro Phe Ser Glu Val Pro Glu Ala Phe Leu Lys Val Glu Arg Gly His Ala Arg Gly Lys Thr Val Val Asn Val Val.

8. A polypeptide having the amino acid sequence of SEQ ID NO: 2 or 4 or a functional derivative of said polypeptide, said functional derivative binding at least one of NAD^+, IL-2R β-chain, or IL-2Rγ-chain.

9. The functional derivative of claim 8, wherein said functional derivative is a variant, a fragment, a chemical derivative, or a fusion protein of said polypeptide.

10. A nucleic acid molecule containing the coding information for a polypeptide or functional derivative according to any one of claims 1 to 9

11. A nucleic acid molecule containing the nucleotide sequence according to SEQ ID NO: 1:

AGAATGGACA GAATACTGAC TGGAACGTTA ATTCGAGCAT TTCATATGCG
AAGAGCGGAA TAACAGTTCC GTATTCTTCT TTCAGTTTCT CCATTAGATT
AGCTTCATTT TCGAAGGCTC CGTTTTGCAT GCTTAATTTT GAAACTAGCC
CGTGGTTTGG CAGAATTTGA CTGAATTCAG GGGTGAGAGT TTGATCCAGT
CCAAGTGTAT TTGAATTTGA GCACGCAGTT CAACCAGTGT TTACA
ATG GAA TTT CTG AAG ACT TGT GTA CTT AGA AGA AAT GCA TGC ACT
GCG GTT TGC TTC TGG AGA AGC AAA GTT GTC CAA AAG CCT TCA GTT
AGA AGG ATT AGT ACT ACC TCT CCA AGG AGC ACT GTC ATG CCT GCT
TGG GTG ATA GAT AAA TAT GGG AAG AAT GAA GTG CTT CGA TTC ACT
CAG AAC ATG ATG ATG CCT ATT ATA CAC TAT CCA AAT GAA GTC ATT
GTC AAA GTT CAC GCT GCC AGT GTA AAT CCT ATA GAC GTT AAT ATG
AGA AGT GGT TAT GGA GCT ACA GCT TTA AAT ATG AAG CGT GAT CCT
TTA CAC GTG AAA ATC AAA GGA GAA GAA TTT CCT CTG ACT CTG GGT
CGG GAT GTC TCT GGC GTG GTG ATG GAA TGT GGG CTT GAT GTG AAA
TAC TTC AAG CCT GGA GAT GAG GTC TGG GCT GCA GTT CCT CCT TGG
AAA CAA GGC ACT CTT TCA GAG TTT GTT GTA GTC AGT GGG AAT GAG
GTC TCT CAC AAA CCC AAA TCA CTC ACT CAT ACT CAA GCT GCC TCT
TTG CCA TAT GTG GCT CTC ACA GCC TGG TCT GCT ATA AAC AAA GTT
GGT GGC CTG AAT GAC AAG AAT TGC ACA GGA AAA CGT GTT CTA ATC
TTA GGC GCT TCA GGC GGA GTT GGT ACT TTT GCT ATA CAG GTA ATG
AAA GCA TGG GAT GCT CAT GTG ACA GCA GTT TGC TCC CAA GAT GCC

AGT GAA CTT GTA AGG AAG CTT GGT GCA GAC GAT GTA ATT GAT TAC
AAA TCT GGA AGT GTG GAA GAG CAG TTG AAA TCC TTA AAA CCA TTT
GAT TTT ATC CTT GAT AAT GTT GGC GGA TCC ACT GAA ACA TGG GCT
CCA GAT TTT CTC AAG AAA TGG TCA GGA GCC ACC TAT GTG ACT TTG
GTG ACT CCT TTC CTC CTG AAC ATG GAC CGA TTG GGC ATA GCA GAT
GGC ATG TTG CAG ACA GGA GTC ACT GTA GGT TCA AAG GCA TTA AAG
CAT TTC TGG AAA GGA GTC CAT TAT CGC TGG GCA TTT TTC ATG GCC
AGT GGC CCA TGT TTA GAT GAC ATT GCA GAA CTG GTG GAT GCG GGA
AAG ATC CGG CCA GTT ATT GAA CAA ACC TTT CCT TTT TCT AAA GTT
CCA GAA GCC TTC CTG AAG GTG GAA AGA GGA CAC GCA CGA GGA AAG
ACT GTA ATT AAT GTT GTT TAAATAAAAA TGCAGTTTAG TGATTAAAAA
AAAAAAAAAA AAAAAAAA,

or a degenerate variant of said nucleic acid molecule, or a nucleic acid molecule capable of hybridizing to a nucleic acid molecule having said nucleotide sequence, or a nucleic acid molecule containing a part of SEQ ID NO:1 or a part of any of the aforementioned nucleic acid sequences, preferably a nucleic acid molecule containing a nucleotide sequence according to SEQ ID NO: 9:

ATG GAA TTT CTG AAG ACT TGT GTA CTT AGA AGA AAT GCA TGC ACT
GCG GTT TGC TTC TGG AGA AGC AAA GTT GTC CAA AAG CCT TCA GTT
AGA AGG ATT AGT ACT ACC TCT CCA AGG AGC ACT GTC ATG CCT GCT
TGG GTG ATA GAT AAA TAT GGG AAG AAT GAA GTG CTT CGA TTC ACT
CAG AAC ATG ATG ATG CCT ATT ATA CAC TAT CCA AAT GAA GTC ATT
GTC AAA GTT CAC GCT GCC AGT GTA AAT CCT ATA GAC GTT AAT ATG
AGA AGT GGT TAT GGA GCT ACA GCT TTA AAT ATG AAG CGT GAT CCT
TTA CAC GTG AAA ATC AAA GGA GAA GAA TTT CCT CTG ACT CTG GGT
CGG GAT GTC TCT GGC GTG GTG ATG GAA TGT GGG CTT GAT GTG AAA
TAC TTC AAG CCT GGA GAT GAG GTC TGG GCT GCA GTT CCT CCT TGG
AAA CAA GGC ACT CTT TCA GAG TTT GTT GTA GTC AGT GGG AAT GAG
GTC TCT CAC AAA CCC AAA TCA CTC ACT CAT ACT CAA GCT GCC TCT
TTG CCA TAT GTG GCT CTC ACA GCC TGG TCT GCT ATA AAC AAA GTT
GGT GGC CTG AAT GAC AAG AAT TGC ACA GGA AAA CGT GTT CTA ATC
TTA GGC GCT TCA GGC GGA GTT GGT ACT TTT GCT ATA CAG GTA ATG
AAA GCA TGG GAT GCT CAT GTG ACA GCA GTT TGC TCC CAA GAT GCC
AGT GAA CTT GTA AGG AAG CTT GGT GCA GAC GAT GTA ATT GAT TAC
AAA TCT GGA AGT GTG GAA GAG CAG TTG AAA TCC TTA AAA CCA TTT
GAT TTT ATC CTT GAT AAT GTT GGC GGA TCC ACT GAA ACA TGG GCT

CCA GAT TTT CTC AAG AAA TGG TCA GGA GCC ACC TAT GTG ACT TTG
GTG ACT CCT TTC CTC CTG AAC ATG GAC CGA TTG GGC ATA GCA GAT
GGC ATG TTG CAG ACA GGA GTC ACT GTA GGT TCA AAG GCA TTA AAG
CAT TTC TGG AAA GGA GTC CAT TAT CGC TGG GCA TTT TTC ATG GCC
AGT GGC CCA TGT TTA GAT GAC ATT GCA GAA CTG GTG GAT GCG GGA
AAG ATC CGG CCA GTT ATT GAA CAA ACC TTT CCT TTT TCT AAA GTT
CCA GAA GCC TTC CTG AAG GTG GAA AGA GGA CAC GCA CGA GGA AAG
ACT GTA ATT AAT GTT GTT.

or a degenerate variant of SEQ ID NO: 9.

12. A nucleic acid molecule according to claim 11, said nucleic acid molecule being capable of hybridizing to a nucleic acid molecule having the nucleotide sequence according to SEQ ID NO: 1 under conditions which select for a homology of more than 70%, more preferably more than 90%.

13. The nucleic acid molecule of claim 12, said nucleic acid molecule being capable of hybridizing in 3 x SSC at 65°C to a nucleic acid molecule containing a nucleotide sequence according to SEQ ID NO: 1.

14. A polypeptide coded by a nucleic acid molecule according to any one of claims 11 to 13.

15. A vector containing the nucleotide sequence of a nucleic acid molecule according to any one of claims 10 to 13.

16. The vector of claim 15, said vector being an expression vector.

17. A host cell carrying a vector according to claims 15 or 16.

18. Method of production of a polypeptide according to any one of claims 1 to 9 or 14, comprising the steps of

a) cultivating a host cell according to claim 17 under conditions where said polypeptide is expressed by said host cell, and

b) isolating said polypeptide.

19. The method of claim 18, wherein said host cell is an *E. coli* cell or a mammalian cell, preferably a COS cell.

20. An antibody molecule specific for a polypeptide or functional derivative according to any one of claims 1 to 9.

21. The antibody molecule of claim 20 which is polyclonal antibody, a monoclonal antibody, a complete immunglobuline or a fragment thereof, the Fab' or $F(ab)_2$ fragment of an immunglobuline, a recombinant antibody or a recombinant antibody fragment, a recombinant single-chain antibody (scFv), a chimeric, bispecific or humanised antibody.

22. The antibody molecule of claim 20 or 21, said antibody molecule being specific for any one of the following amino acid sequences:

SEQ ID NO:10:	CKVVQKPSVRRISTTSPRST,
SEQ ID NO:11:	CYKSGSVEEQLKSLKPFDFI,
SEQ ID NO:12:	CGGSTETWAPDFLKKWSGAT.

23. An antisense oligonucleotide corresponding to a part of the sequence of a nucleic acid molecule according to any one of claims 10 to 13.

24. The antisense oligonucleotide of claim 23 which has the nucleic acid sequence SEQ ID NO: 8: 5'-GTCTTCAAAACGCCCATCCT-3'.

25. The use of an antisense nucleotide according to claims 23 or 24 for the inhibition of IL-2 dependent cell growth.

26. A pharmaceutical composition containing an antisense nucleotide according to claims 23 or 24.

27. A pharmaceutical composition containing a polypeptide according to any one of claims 1 to 9, or 14.

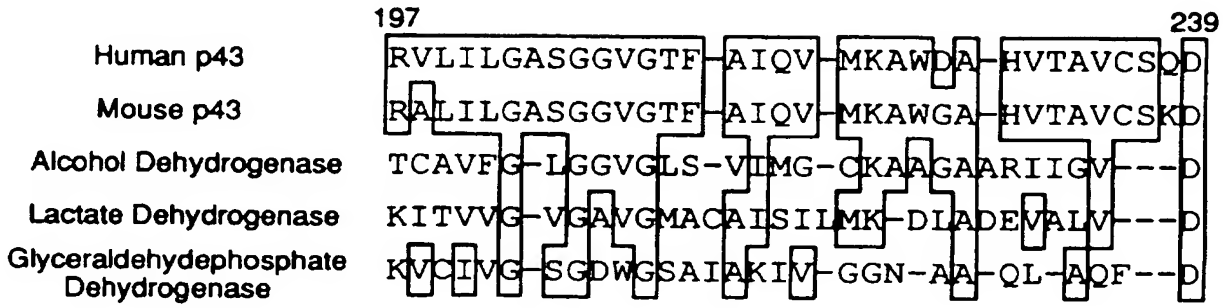
28. A pharmaceutical composition containing a nucleic acid molecule according to any one of claims 10 to 13, 15 or 16.

```
    AGAATGGACAGAATACTGACTGGAACGTTAATTCGAGCATTTCATATGCGAAGAGCGGAATAACAGTTCCGTATTCTTC
    TTTCAGTTTCTCCATTAGATTAGCTTCATTTTCGAAGGCTCCGTTTTGCATGCTTAATTTTGAAACTAGCCCGTGGTTT
    GGCAGAATTTGACTGAATTCAGGGGTGAGAGTTTGATCCAGTCCAAGTGTATTTGAATTTGAGCACGCAGTTCAACCAG
    TGTTTACA
                    M   E   F   L   K   T   C   V   L   R   R   M   A   C   T   A   V     17
246                 ATG GAA TTT CTG AAG ACT TGT GTA CTT AGA AGA AAT GCA TGC ACT GCG GTT

    C   F   W   R   S   K   V   V   Q   K   P   S   V   R   R   I   S   T   T   S     37
297 TGC TTC TGG AGA AGC AAA GTT GTC CAA AAG CCT TCA GTT AGA AGG ATT AGT ACT ACC TCT

    P   R   S   T   V   M   P   A   W   V   I   D   K   Y   G   K   N   E   V   L     57
357 CCA AGG AGC ACT GTC ATG CCT GCT TGG GTG ATA GAT AAA TAT GGG AAG AAT GAA GTG CTT

    R   F   T   Q   N   M   M   M   P   I   I   N   Y   P   N   E   V   I   V   K     77
417 CGA TTC ACT CAG AAC ATG ATG ATG CCT ATT ATA CAC TAT CCA AAT GAA GTC ATT GTC AAA

    V   N   A   A   S   V   N   P   I   D   V   N   M   R   S   G   Y   G   A   T     97
477 GTT CAC GCT GCC AGT GTA AAT CCT ATA GAC GTT AAT ATG AGA AGT GGT TAT GGA GCT ACA

    A   L   N   M   K   R   D   P   L   N   V   K   I   K   G   E   E   F   P   L    117
537 GCT TTA AAT ATG AAG CGT GAT CCT TTA CAC GTG AAA ATC AAA GGA GAA GAA TTT CCT CTG

    T   L   G   R   D   V   S   G   V   V   M   E   C   G   L   D   V   K   Y   F    137
597 ACT CTG GGT CGG GAT GTC TCT GGC GTG GTG ATG GAA TGT GGG CTT GAT GTG AAA TAC TTC

    K   P   G   D   E   V   W   A   A   V   P   P   W   K   Q   G   T   L   S   E    157
657 AAG CCT GGA GAT GAG GTC TGG GCT GCA GTT CCT CCT TGG AAA CAA GGC ACT CTT TCA GAG

    F   V   V   V   S   G   N   E   V   S   N   K   P   K   S   L   T   N   T   Q    177
717 TTT GTT GTA GTC AGT GGG AAT GAG GTC TCT CAC AAA CCC AAA TCA CTC ACT CAT ACT CAA

    A   A   S   L   P   Y   V   A   L   T   A   W   S   A   I   N   K   V   G   G    197
777 GCT GCC TCT TTG CCA TAT GTG GCT CTC ACA GCC TGG TCT GCT ATA AAC AAA GTT GGT GGC

    L   N   D   K   N   C   T   G   K   R   V   L   I   L   G   A   S   G   G   V    217
837 CTG AAT GAC AAG AAT TGC ACA GGA AAA CGT GTT CTA ATC TTA GGC GCT TCA GGC GGA GTT

    G   T   F   A   I   Q   V   M   K   A   W   D   A   N   V   T   A   V   C   S    237
897 GGT ACT TTT GCT ATA CAG GTA ATG AAA GCA TGG GAT GCT CAT GTG ACA GCA GTT TGC TCC

    Q   D   A   S   E   L   V   R   K   L   G   A   D   D   V   I   D   Y   K   S    257
957 CAA GAT GCC AGT GAA CTT GTA AGG AAG CTT GGT GCA GAC GAT GTA ATT GAT TAC AAA TCT

    G   S   V   E   E   Q   L   K   S   L   K   P   F   D   F   I   L   D   N   V    277
1017 GGA AGT GTG GAA GAG CAG TTG AAA TCC TTA AAA CCA TTT GAT TTT ATC CTT GAT AAT GTT

    G   G   S   T   E   T   W   A   P   D   F   L   K   K   W   S   G   A   T   Y    297
1077 GGC GGA TCC ACT GAA ACA TGG GCT CCA GAT TTT CTC AAG AAA TGG TCA GGA GCC ACC TAT

    V   T   L   V   T   P   F   L   L   N   M   D   R   L   G   I   A   D   G   M    317
1137 GTG ACT TTG GTG ACT CCT TTC CTC CTG AAC ATG GAC CGA TTG GGC ATA GCA GAT GGC ATG

    L   Q   T   G   V   T   V   G   S   K   A   L   K   H   F   W   K   G   V   H    337
1197 TTG CAG ACA GGA GTC ACT GTA GGT TCA AAG GCA TTA AAG CAT TTC TGG AAA GGA GTC CAT

    Y   R   W   A   F   F   M   A   S   G   F   C   L   D   D   I   A   E   L   V    357
1257 TAT CGC TGG GCA TTT TTC ATG GCC AGT GGC CCA TGT TTA GAT GAC ATT GCA GAA CTG GTG

    D   A   G   K   I   R   P   V   I   E   Q   T   F   P   F   S   K   V   P   E    377
1317 GAT GCG GGA AAG ATC CGG CCA GTT ATT GAA CAA ACC TTT CCT TTT TCT AAA GTT CCA GAA

    A   F   L   K   V   E   R   G   N   A   R   G   K   T   V   I   N   V   V        396
1377 GCC TTC CTG AAG GTG GAA AGA GGA CAC GCA CGA GGA AAG ACT GTA ATT AAT GTT GTT TAA

1437 ATAAAAATGCAGTTTAGTGATTAAAAAAAAAAAAAAAAAAAAAAAA
```

Fig 1A

```
Human p43 MEFLKTCVLR RNACTAVCFW RSKVVQKPSV RRISTTSPRS TVMPAWVIDK YGKNEVLRFT   60
          |??|||||||| |?|||?|?||| |???|??|?? |?|||||?|| |||||||||| ||||||||||
Mouse p43 MGVLKTCVLR RSACAAACFW RRTVIPKPPF RGISTTSARS TVMPAWVIDK YGKNEVLRFT   60

Human p43 QNMMMPIIHY PNEVIVKVHA ASVNPIDVNM RSGYGATALN MKRDPLHVKI KGEEFPLTLG  120
          ||||?||||| ||||||?|||| |||||||||| |||||||||| |||||||?|? ||||||||||
Mouse p43 QNMMLPIIHY PNEVIIKVHA ASVNPIDVNM RSGYGATALN MKRDPLHMKT KGEEFPLTLG  120

Human p43 RDVSGVVMEC GLDVKYFKPG DEVWAAVPPW KQGTLSEFVV VSGNEVSHKP KSLTHTQAAS  180
          |||||||||| |||||||?|| |||||||||| |||||||||| |||||||||| ||||||||||
Mouse p43 RDVSGVVMEC GLDVKYFQPG DEVWAAVPPW KQGTLSEFVV VSGNEVSHKP KSLTHTQAAS  180

Human p43 LPYVALTAWS AINKVGGLND KNCTGKRVLI LGASGGVGTF AIQVMKAWDA HVTAVCSQDA  240
          |||||||||| |||||||?| ?|||?||?|| |||||||||| |||||||?| ||||||||?||
Mouse p43 LPYVALTAWS AINKVGGLSD RNCKGKRALI LGASGGVGTF AIQVMKAWGA HVTAVCSKDA  240

Human p43 SELVRKLGAD DVIDYKSGSV EEQLKSLKPF DFILDNVGGS TETWAPDFLK KWSGATYVTL  300
          |||||||||| ?|||||??||| |||||||||?? ?|||||||||| ||||||??||| ||||||||||
Mouse p43 SELVRKLGAD EVIDYTLGSV EEQLKSLKLC AFILDNVGGS TETWALNFLK KWSGATYVTL  300

Human p43 VTPFLLNMDR LGIADGMLQT GVTVGSKALK HFWKGVHYRW AFFMASGPCL DDIAELVDAG  360
          |||||||||| ||?|||||||| |||||?|||?| |?|?|||||| |||||||||?| |?|||||||||
Mouse p43 VTPFLLNMDR LGVADGMLQT GVTVGTKAMK HLWQGVHYRW AFFMASGPYL DEIAELVDAG  360

Human p43 KIRPVIEQTF PFSKVPEAFL KVERGHARGK TVINVV                          396
          |||||||?|| |||?|||||| |||||||||| ||?|||
Mouse p43 KIRPVIERTF PFSEVPEAFL KVERGHARGK TVVNVV                          396
```

Fig 1B


197
239
Human p43
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Mouse p43
RALILGASGGVGTF-AIQV-MKAWGA-HVTAVCSKD
Alcohol Dehydrogenase
TCAVFG-LGGVGLS-VIMG-CKAAGAARIIGV---D
Lactate Dehydrogenase
KITVVG-VGAVGMACAISILMK-DLADEVALV---D
Glyceraldehydephosphate Dehydrogenase
KVCIVG-SGDWGSAIAKIV-GGN-AA-QL-AQF--D

Fig 2

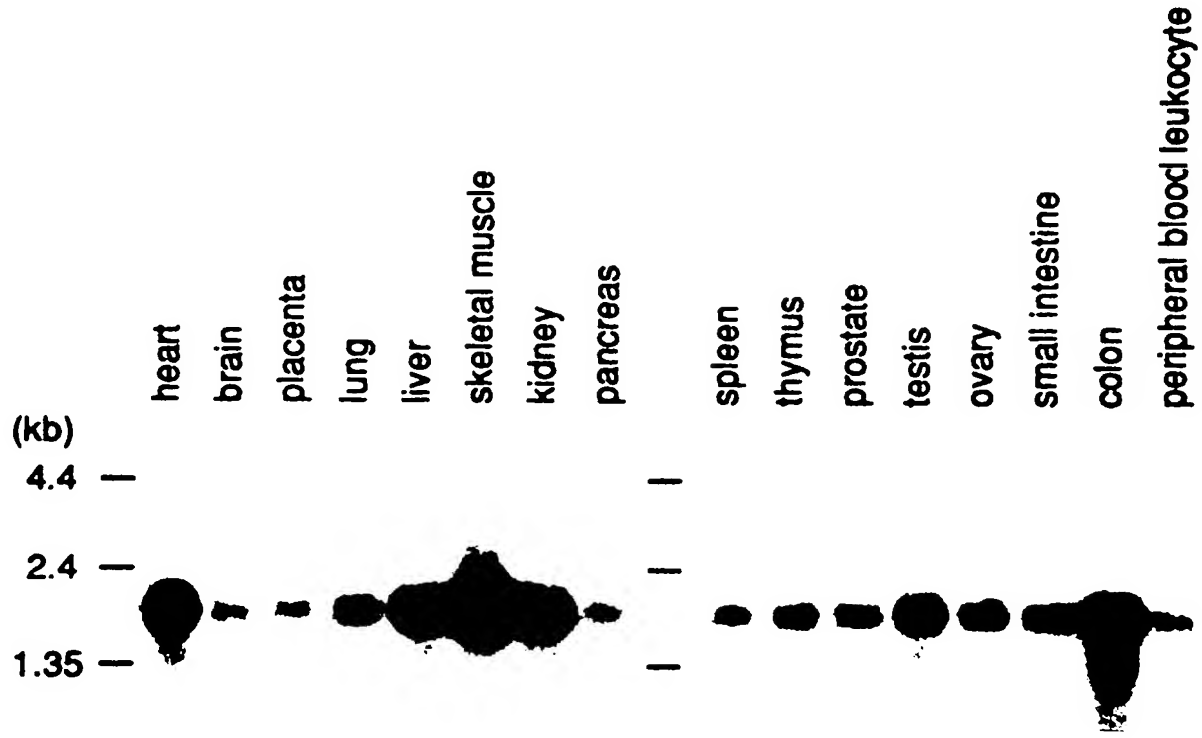

heart
brain
placenta
lung
liver
skeletal muscle
kidney
pancreas
spleen
thymus
prostate
testis
ovary
small intestine
colon
peripheral blood leukocyte
(kb)
4.4
2.4
1.35

Fig 3

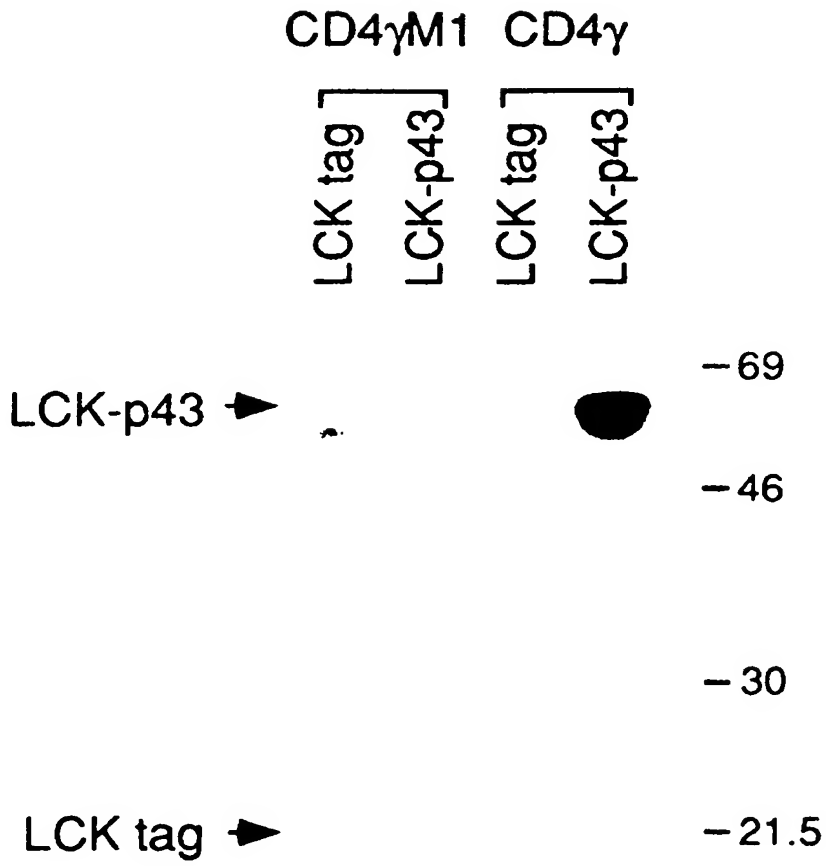

CD4γM1
CD4γ
LCK tag
LCK-p43
LCK tag
LCK-p43
LCK-p43
LCK tag
69
46
30
21.5

Fig 4A

Fig 4B

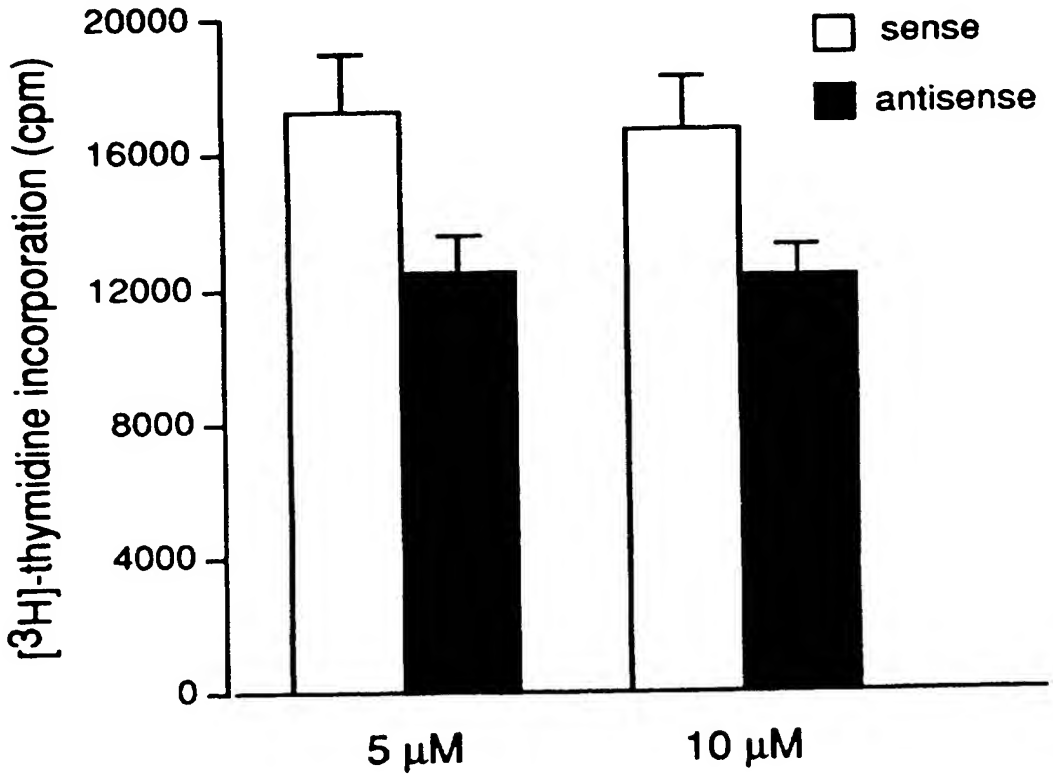

20000
16000
12000
8000
4000
0
[3H]-thymidine incorporation (cpm)
sense
antisense
5 µM
10 µM

Fig 5

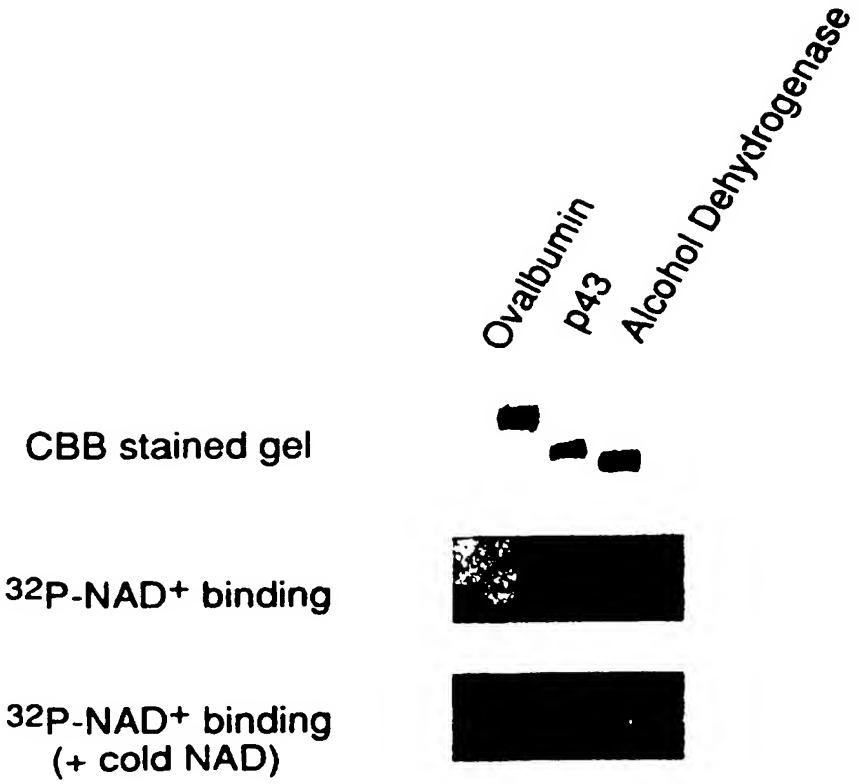

Ovalbumin
p43
Alcohol Dehydrogenase
CBB stained gel
32P-NAD+ binding
32P-NAD+ binding
(+ cold NAD)

Fig 6

INTERNATIONAL SEARCH REPORT

Interna · Application No
PCT/EP 95/05123

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/715 C07K14/47 C12N15/70 C12N15/85
C12N1/20 C12N5/10 C07K16/28 A61K31/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category ·	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 621 338 (AJINOMOTO) 26 October 1994 see claims -----	1

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

· Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
16 April 1996	23.04.96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Delanghe, L

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 95/05123

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons.

1. [X] Claims Nos.: 25
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: As far as claim 25 is directed to a method of treatment of the human body the search has been carried out and based on the alleged effects of the compound.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Interna ' Application No
PCT/EP 95/05123

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-621338	26-10-94	JP-A- 7313188	05-12-95

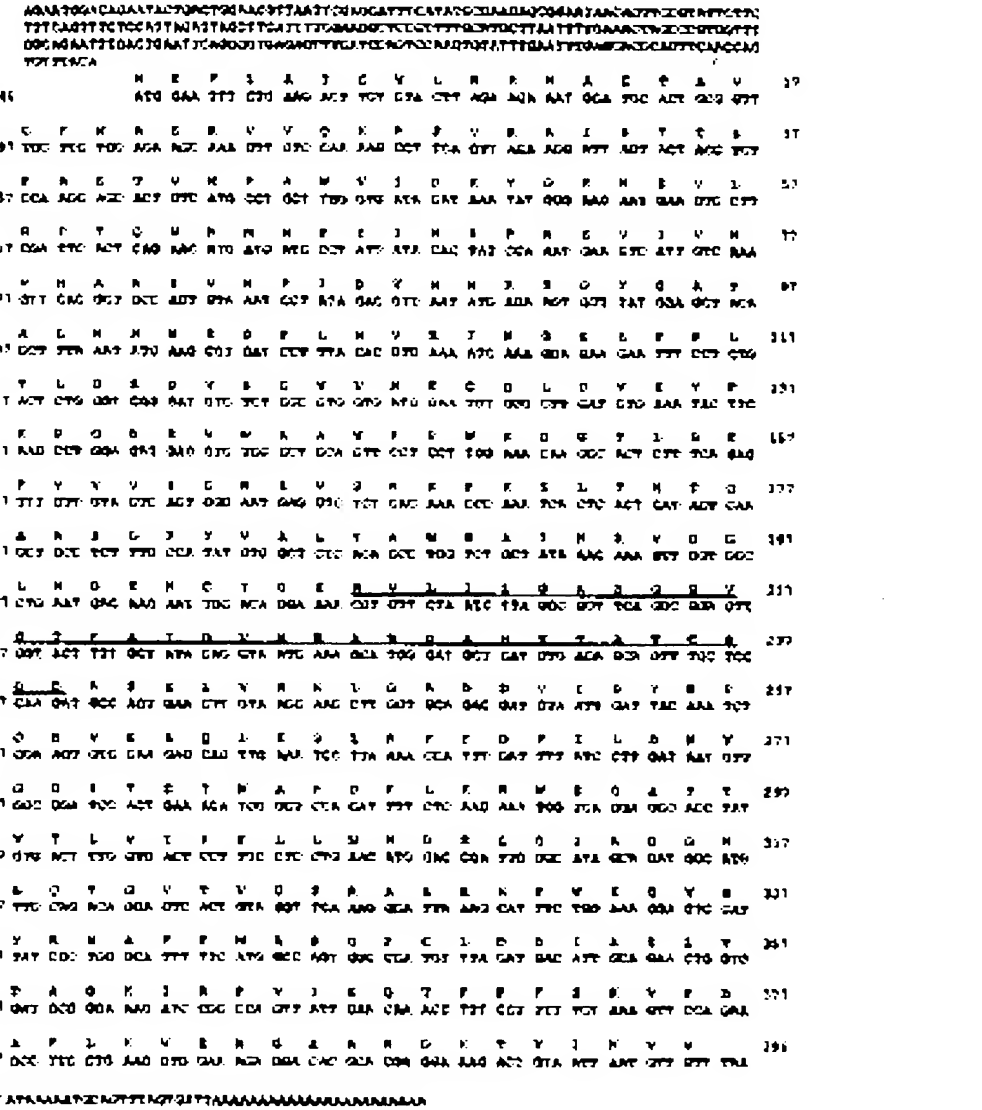
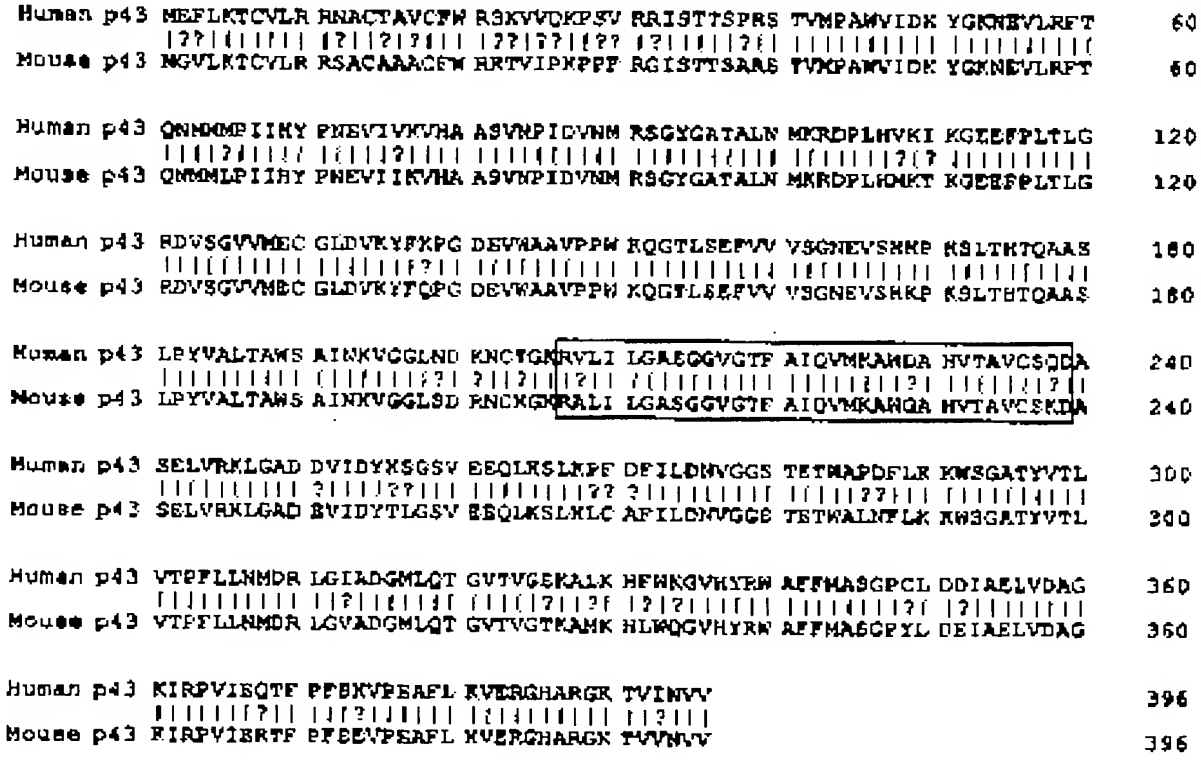


Fig 1A


Human p43 MEFLKTCVLR RNACTAVCFW RSKVVQKPSV RRISTTSPRS TVMPAMVIDK YGKNEVLRFT 60
Mouse p43 MGVLKTCVLR RSACAAACFW RRTVIPKPPF RGISTTSAAS TVKPAMVIDK YGKNEVLRFT 60
Human p43 QNNKMPIIKY PNEVIVKVHA ASVNPIDVNM RSGYGATALN MKRDPLHVKI KGLEFPLTLG 120
Mouse p43 QNMMLPIIHY PNEVIIKVHA ASVNPIDVNM RSGYGATALN MKRDPLHMKT KGEEFPLTLG 120
Human p43 RDVSGVVMEC GLDVKYFKPG DEVWAAVPPW KQGTLSEFVV VSGNEVSRKP KSLTHTQAAS 180
Mouse p43 RDVSGVVMEC GLDVKYFQPG DEVWAAVPPW KQGTLSEFVV VSGNEVSRKP KSLTHTQAAS 180
Human p43 LPYVALTAWS AINKVGGLND RNCYGKRVLI LGASGGVGTF AIQVMKAWDA HVTAVCSQDA 240
Mouse p43 LPYVALTAWS AINKVGGLSD RNCKGKRALI LGASGGVGTF AIQVMKAWQA HVTAVCSKDA 240
Human p43 SELVRKLGAD DVIDYKSGSV EEQLKSLKPF DFILDNVGGS TETWAPDFLK KWSGATYVTL 300
Mouse p43 SELVRKLGAD EVIDYTLGSV EEQLKSLKLC AFILDNVGGS TETWALNFLK KWSGATYVTL 300
Human p43 VTPFLLNMDR LGIADGMLQT GVTVGSKALK HFWKGVHYRW AFFMASGPCL DDIAELVDAG 360
Mouse p43 VTPFLLNMDR LGVADGMLQT GVTVGTKAMK HLWQGVHYRW AFFMASGPYL DEIAELVDAG 360
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Mouse p43 KIRPVIERTF PFEEVPEAFL KVERGHARGK TVVNVV 396

Fig 1B

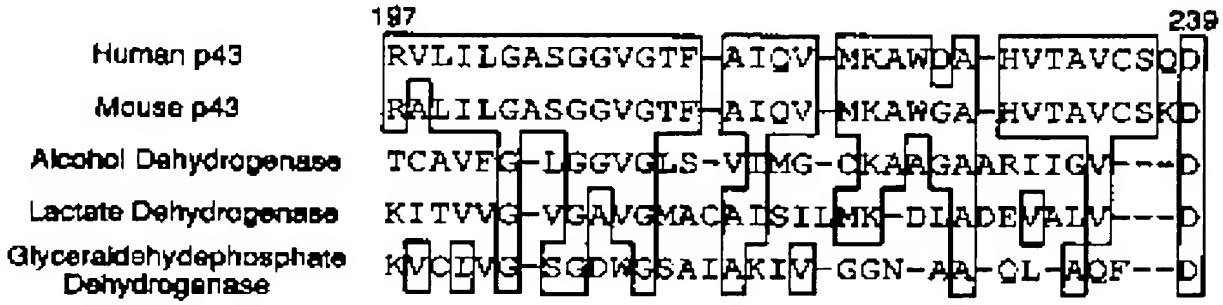
197
239
Human p43
Mouse p43
Alcohol Dehydrogenase
Lactate Dehydrogenase
Glyceraldehydephosphate Dehydrogenase

Fig 2

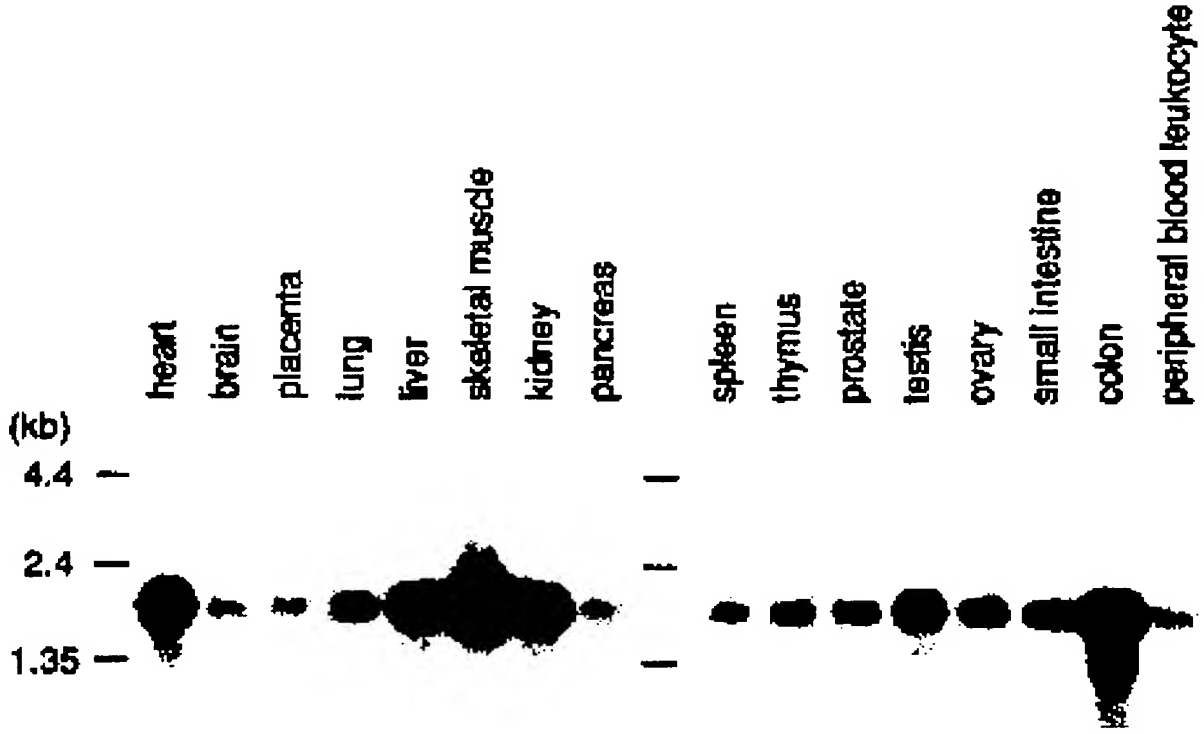
heart
brain
placenta
lung
liver
skeletal muscle
kidney
pancreas
spleen
thymus
prostate
testis
ovary
small intestine
colon
peripheral blood leukocyte
(kb)
4.4
2.4
1.35

Fig 3

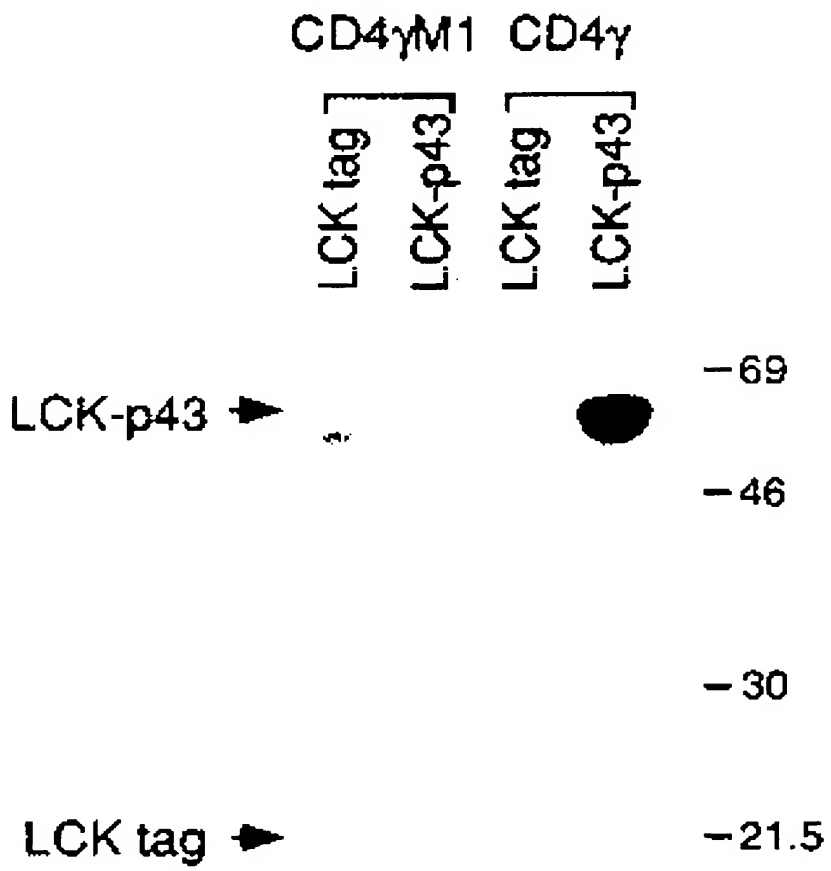
CD4γM1
CD4γ
LCK tag
LCK-p43
LCK tag
LCK-p43
LCK-p43
LCK tag
69
46
30
21.5

Fig 4A

Fig 4B

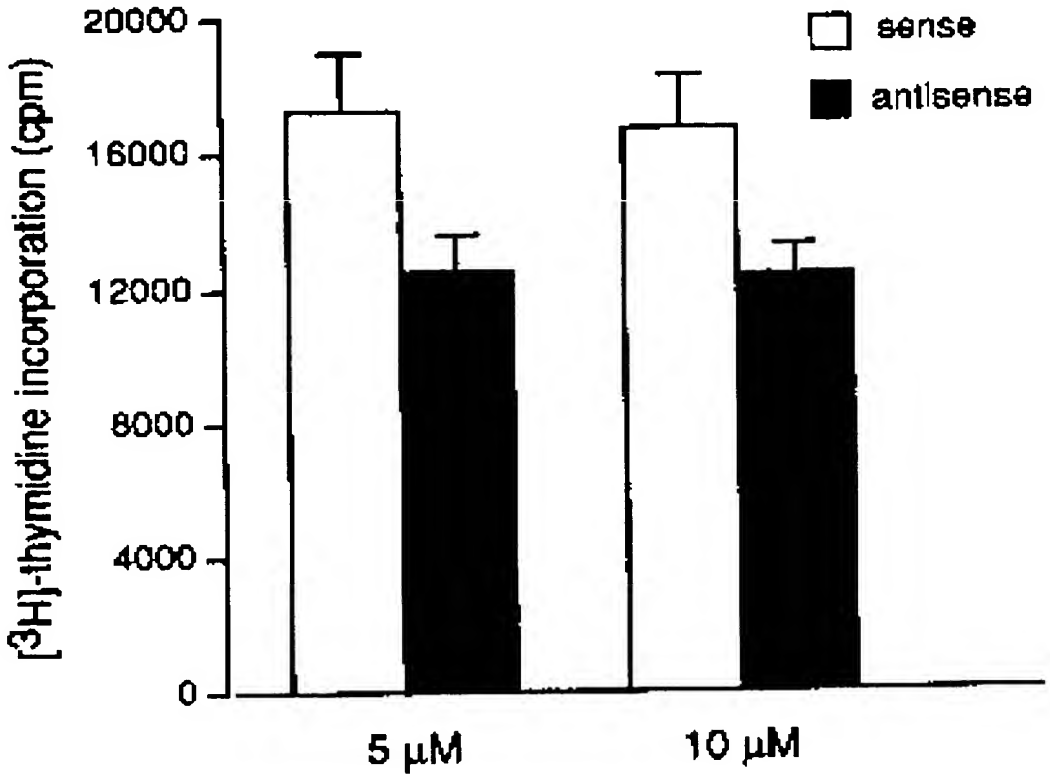
20000
16000
12000
8000
4000
0
[3H]-thymidine incorporation (cpm)
sense
antisense
5 µM
10 µM

Fig 5

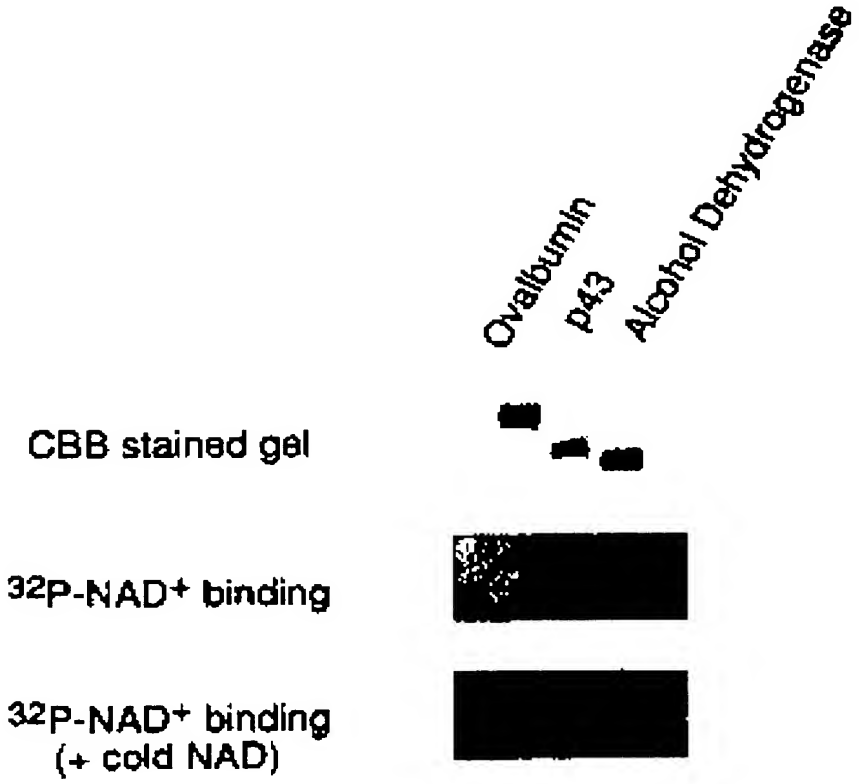
Ovalbumin
p43
Alcohol Dehydrogenase
CBB stained gel
32P-NAD+ binding
32P-NAD+ binding
(+ cold NAD)

Fig 6